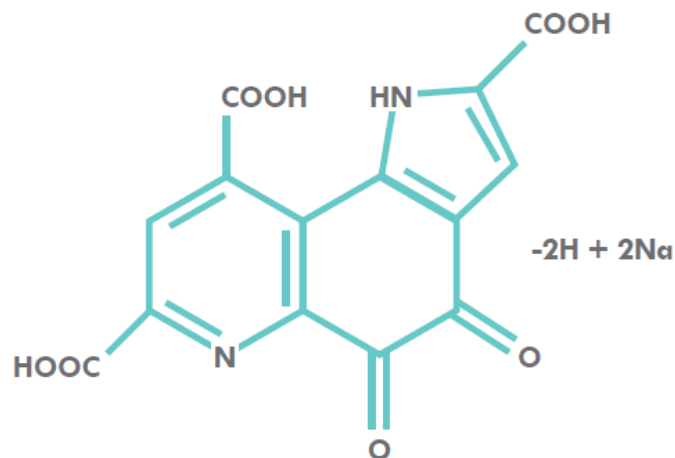


PQQ —
A Novel Human Essential Nutrient

General Information of PQQ



Stable small molecular

Chemical Name: Pyrroloquinoline Quinone Disodium Salt (PQQ•Na₂)

Molecular Formula: C₁₄H₄N₂Na₂O₈

Molecular Weight: 374.17

CAS Number: 122628-50-6

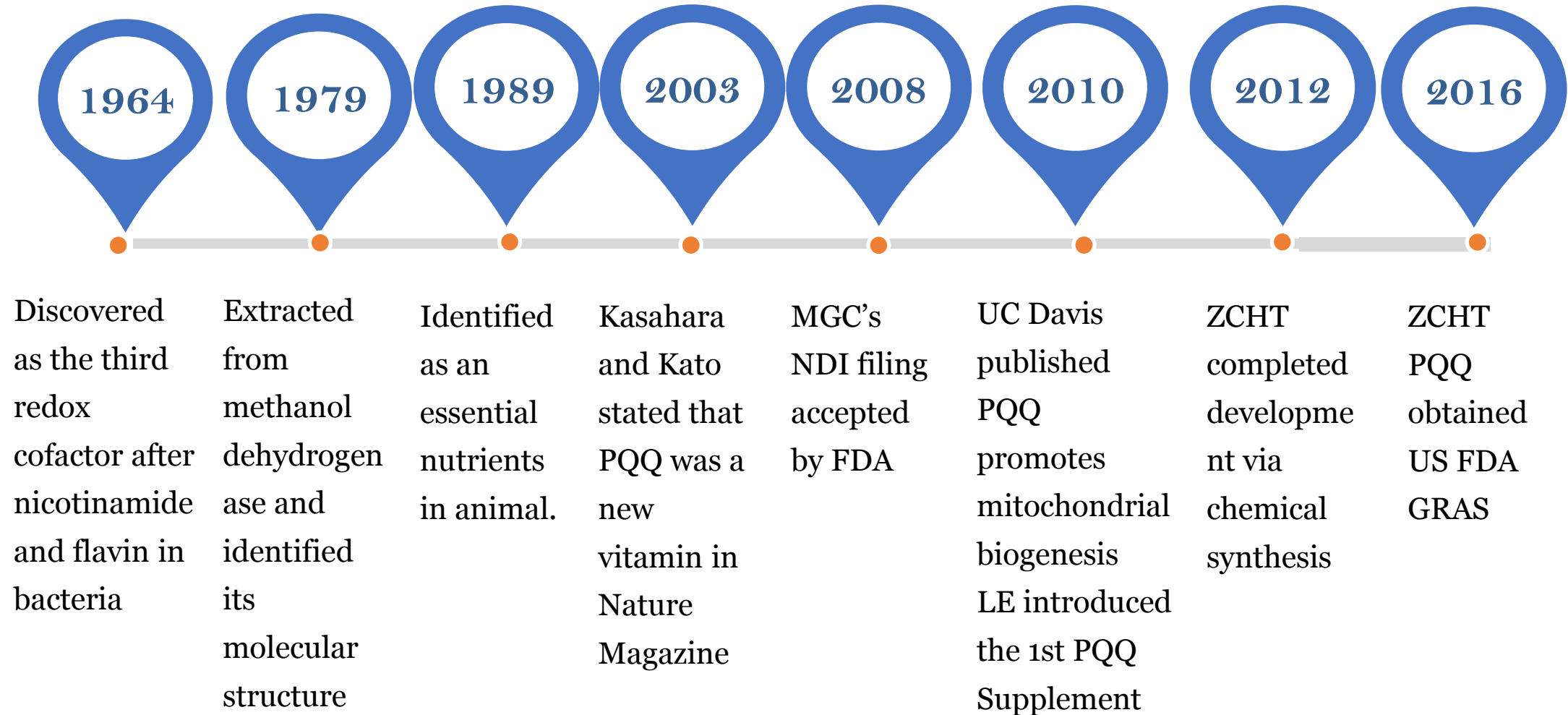
Appearance: Reddish brown powder

Melting Point: >300°C (decomposed during the assay)

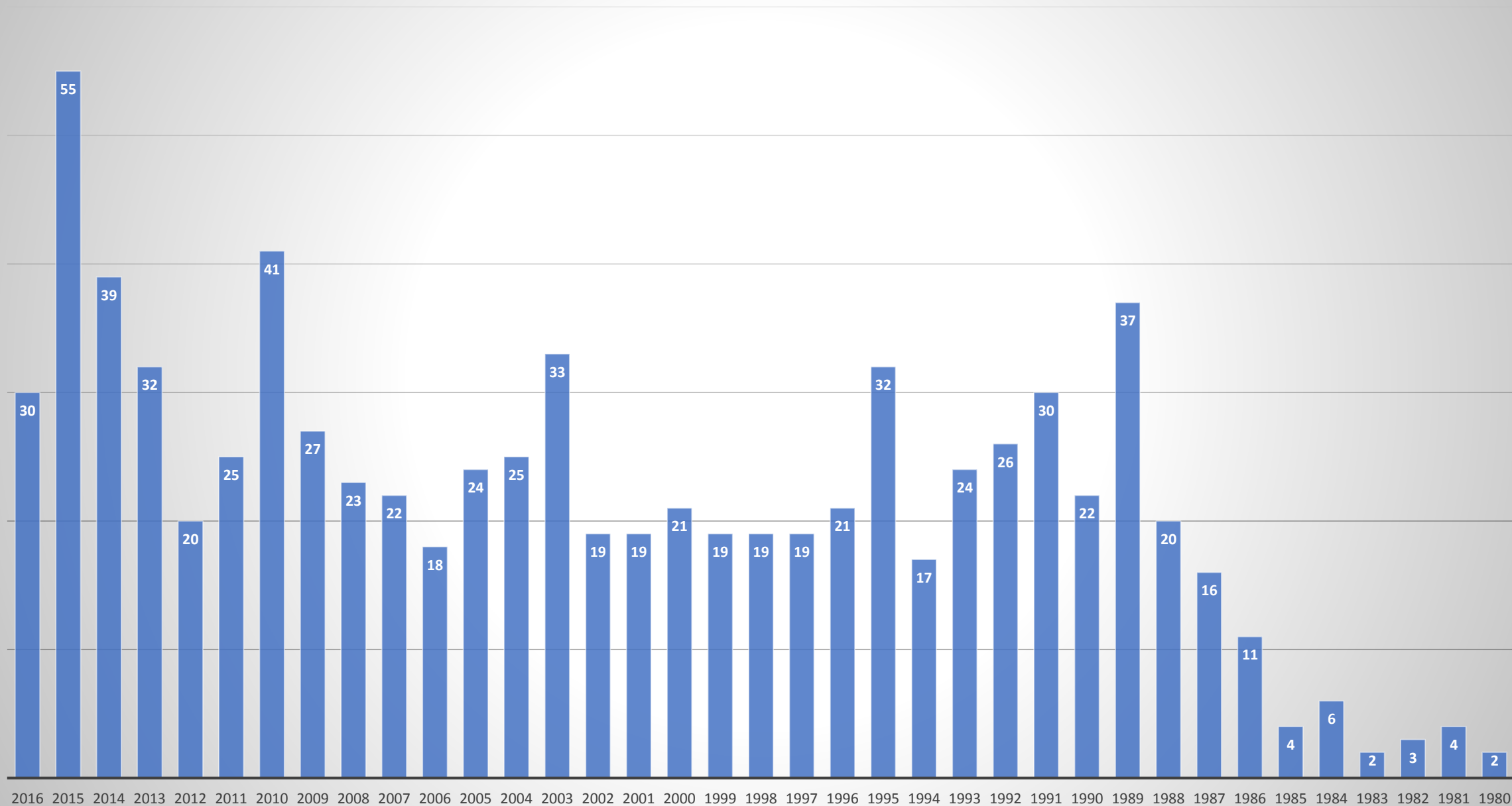
Solubility: Water-soluble (3g/L at 25°C)

Stability: Stable for at least 24 months.

50+ Years of Research



More than 800 studies on PQQ published (by July 2016)



The Discovery of PQQ



Produced by Bacteria



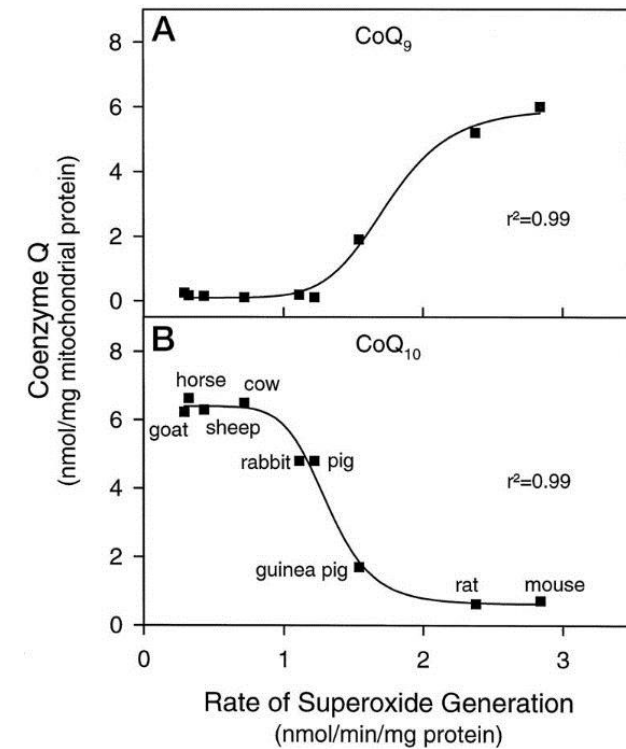
Plants Growth Factor



Playing important roles in Mammals



PQQ is Widely Distributed in Nature



PQQ is Widely Distributed in Daily Life

<i>Food</i>	<i>PQQ(ng/g)</i>	<i>Food</i>	<i>PQQ(ng/g or ml)</i>
<i>Plant</i>		<i>Beverage</i>	
<i>Broad Bean</i>	<i>17.8 ±6.8</i>	<i>Green Tea</i>	<i>29.6 ±12.9</i>
<i>Soy Bean</i>	<i>9.3 ±3.8</i>	<i>Oolong Tea</i>	<i>27.7 ±1.9</i>
<i>Patato</i>	<i>16.6 ±7.3</i>	<i>Whisky</i>	<i>7.9 ±1.8</i>
<i>Sweet Potato</i>	<i>13.3 ±3.7</i>	<i>Wine</i>	<i>5.8 ±2.7</i>
<i>Celery</i>	<i>34.2 ±11.6</i>	<i>Saka</i>	<i>3.7 ±1.4</i>
<i>Cabbage</i>	<i>16.8 ±2.8</i>	<i>Fermented food and others</i>	
<i>Green Pepper</i>	<i>28.2 ±13.7</i>	<i>Natto</i>	<i>61.0 ±31.3</i>
<i>Spinach</i>	<i>21.9 ±6.2</i>	<i>Sauce</i>	<i>16.7 ±3.3</i>
<i>Tomato</i>	<i>9.2 ±1.8</i>	<i>Tofu</i>	<i>24.4 ±12.5</i>
<i>Apple</i>	<i>6.1 ±1.4</i>	<i>Breast Milk</i>	<i>140-180*</i>

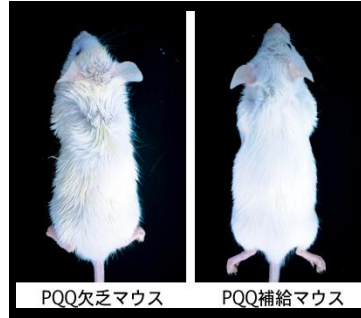
The PQQ content of food products listed is substantially lower than the content of supplemented PQQ (10-20mg) and food ingestion is unlikely to replicate the effects of supplementation due to the magnitude of difference

16mg PQQ =
100L/25G milk

PQQ Deficiency (1989)

Mice fed with PQQ-deficient diet

- Compromised immunity
- Impaired reproductive capability
- Friable skin
- Fewer mitochondria in their tissue
- Rates of conception, the number of offspring, and survival rates in juvenile animals are also significantly reduced in the absence of PQQ.
- When PQQ is introduced back into the diet, it reverses these effects, restoring systemic function while increasing mitochondrial number and energy efficiency simultaneously



PQQ is associated with mitochondrial functions and quantities.

Science. 1989 Aug 25;245(4920):850-2



Biofactors in food play a role in enhancing mitochondrial function, thereby decreasing the risk of some chronic diseases. *Top*, a mouse that has been deprived of pyrroloquinoline quinone (PQQ), a ubiquitous bacterial compound found in fermented products, tea, cocoa and legumes. *Above*, a mouse fed a diet containing PQQ.

[Science](#). 1989 Aug 25;245(4920):850-2.

Nutritional importance of pyrroloquinoline quinone.

[Killgore J](#)¹, [Smidt C](#), [Duich L](#), [Romero-Chapman N](#), [Tinker D](#), [Reiser K](#), [Melko M](#), [Hyde D](#), [Rucker RB](#).

Mice fed a chemically defined diet devoid of pyrroloquinoline quinone (PQQ) grew poorly, failed to reproduce, and became osteolathyrptic. Moreover, severely affected mice had friable skin, skin collagen that was readily extractable into neutral salt solutions, and decreased lysyl oxidase.

The identification of functional defects in connective tissue and the growth retardation associated with PQQ deprivation suggest that **PQQ plays a fundamental role as a growth factor or vitamin.**

[Proc Soc Exp Biol Med](#). 1991 May;197(1):19-26.

Physiologic importance of pyrroloquinoline quinone.

[Smidt CR](#)¹, [Steinberg FM](#), [Rucker RB](#).

Pyrroloquinoline quinone (PQQ, methoxatin) is a dissociable cofactor for a number of bacterial dehydrogenases. The compound is unusual because of its ability to catalyze redox cycling reactions at a high rate of efficiency and it has the potential of catalyzing various carbonyl amine reactions as well. In methylotrophic bacteria, PQQ is derived from the condensation of L-tyrosine with L-glutamic acid.

Whether or not PQQ serves as a cofactor in higher plants and animals remains controversial.

Nevertheless, a strong case may be made that PQQ and related quinoids have nutritional and pharmacologic importance. In highly purified, chemically defined diets, **PQQ stimulates animal growth.**

Furthermore, **PQQ deprivation appears to impair connective tissue maturation**, particularly when initiated in utero and throughout perinatal development.

Japanese Find 1st New Vitamin in 55 Years; Experiments Show It Could Affect Fertility

Byline: Jennifer Harper, [The Washington Times \(Washington, DC\), April 28, 2003](#)

- Japanese researchers say they've discovered the first genuinely "new" vitamin in 55 years and it may prove to be a fertility enhancer. This could cause considerable hubbub in the billion-dollar vitamin supplement industry.
- Pyrroloquinoline quinone, or PQQ, is a member of the B-vitamin group, the researchers explain. But in a statement released Friday, the Tokyo-based Institute of Physical and Chemical Research announced it had studied one particular effect of PQQ on mice.
- Those deprived of it had markedly lowered fertility and "roughened fur," according to project director Takafumi Kato. PQQ played "an important role" in fertility, he said, adding that humans usually react much like rodents to such substances. ...

A New Redox-cofactor Vitamin (2003)

brief communications

Nutritional biochemistry

A new redox-cofactor vitamin for mammals

Nicotinamides and flavins are essential cofactors in enzyme-catalysed reduction-oxidation (redox) reactions and are classified as vitamins because they must be supplied in the diet. Another redox

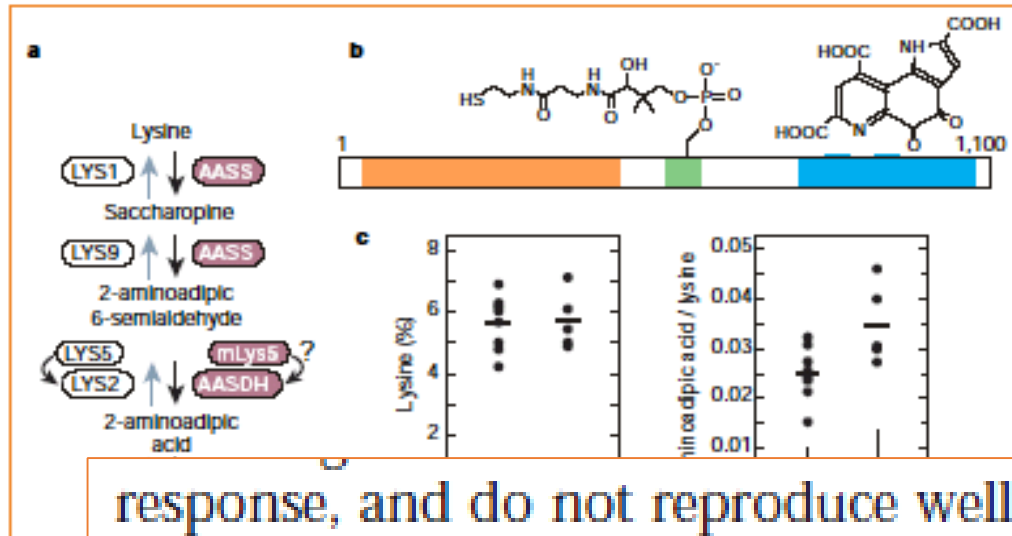
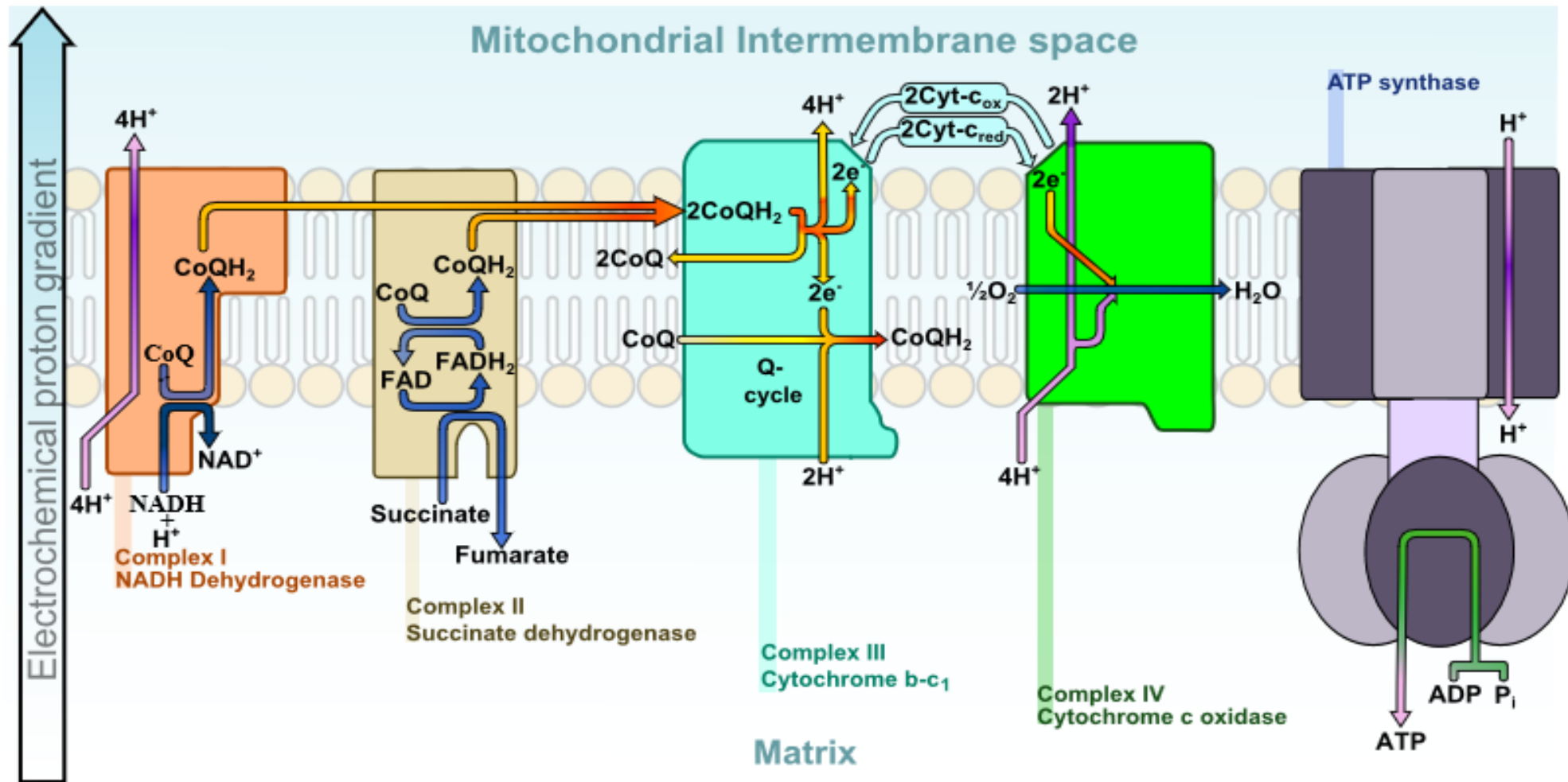


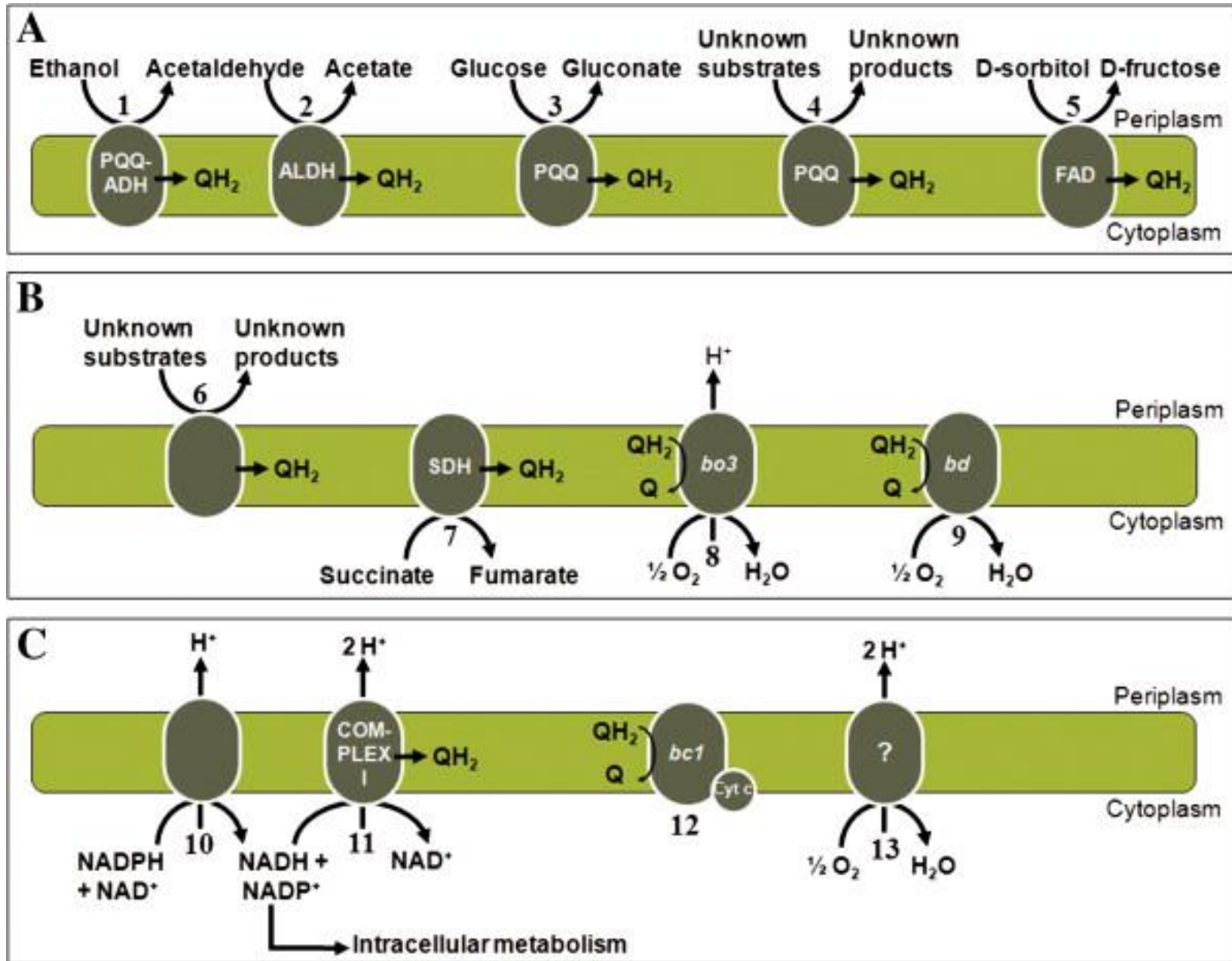
Figure 1
lysine degradation
in a reaction
b, Arrangement
and PQQ-lysine
pentathione
c, Peripheral
as a percentage
respect to

response, and do not reproduce well^{2,3}. On the basis of our demonstration of its molecular function, we propose that PQQ should be classified as a new B vitamin, joining niacin/nicotinic acid (vitamin B3) and riboflavin (vitamin B2), the redox-cofactor derivatives of which are $\text{NAD}^+/\text{NADP}^+$ and FAD/FMN , respectively.

Pyrroloquinoline quinone (PQQ) is the third redox cofactor after nicotinamide and flavin



CoQ is a critical component of the mitochondrial electron transport chain where it shuttles electrons from complexes I and II to complex III. In addition to its vital role in cellular respiration, CoQ is instrumental in cellular antioxidation, extracellular electron transport, and membrane rigidity.



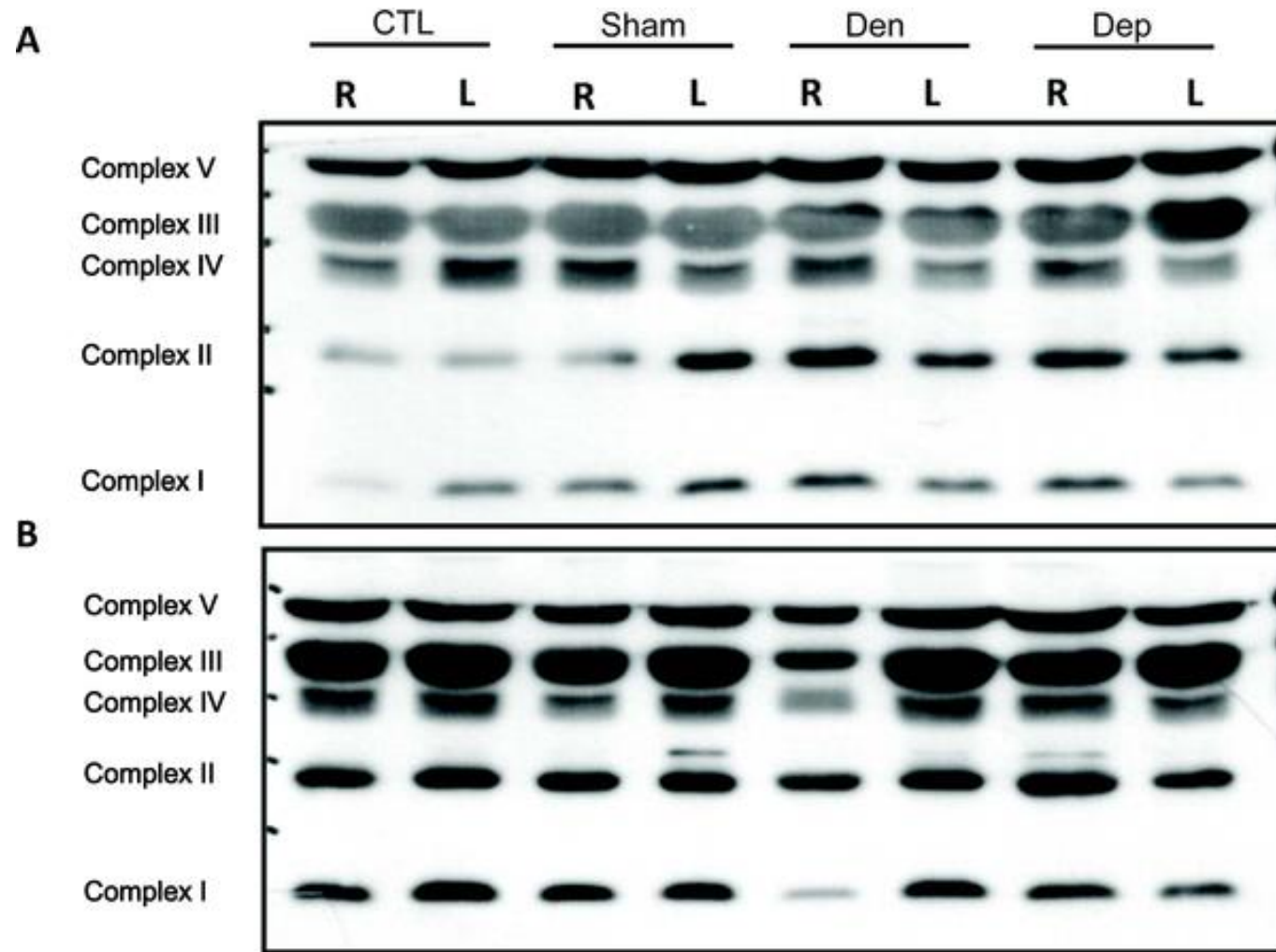
Respiratory chain of *Acetobacter pasteurianus* 386B.

(A) Membrane-bound PQQ- and FAD-dependent **dehydrogenases (complex II)**: (1) PQQ-dependent **alcohol** dehydrogenase; (2) membrane-bound acetaldehyde dehydrogenase; (3) PQQ-dependent **glucose** dehydrogenase; (4) uncharacterized PQQ-containing oxidoreductases; (5) FAD-dependent **sorbitol** dehydrogenase.

(B) Membrane-bound oxidoreductases and terminal oxidases: (6) uncharacterized oxidoreductases; (7) **succinate** dehydrogenase/**fumarate** reductase; (8) cytochrome bo3 ubiquinol oxidase; (9) cytochrome bd ubiquinol oxidase.

(C) Respiratory chain core system: (10) proton-translocating transhydrogenase; (11) proton-translocating NADH:ubiquinone oxidoreductase (**complex I**); (12) bc1 complex ubiquinol:cytochrome c oxidoreductase (**complex III**); (13) incomplete cytochrome c oxidase. Cyt c: cytochrome c.

Western blot of OXPHOS mitochondrial complexes.

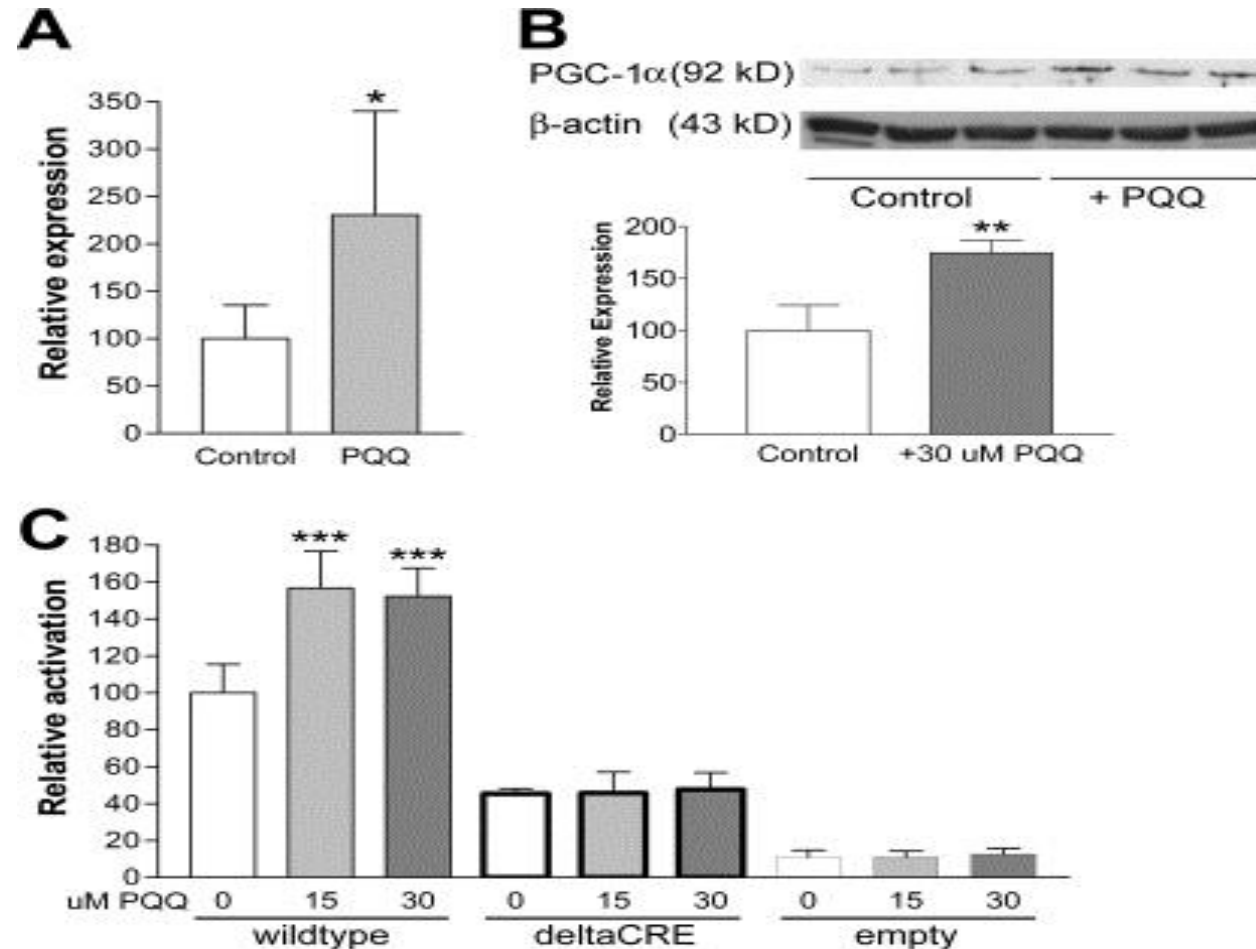


The expression of mitochondrial complexes in skeletal muscle from the control, sham, denervated, and PQQ administered groups were evaluated on the 7th and the 21st days post-denervation, respectively. The OXPHOS complexes exhibited no change on the 7th day after denervation (A). However, the protein levels of all of the OXPHOS complexes except for complex V were significantly ($P < 0.05$) decreased in the right hindlimb on the 21st day after denervation (B). The most dramatic decrease was observed in NADH-TR and COX. However, the protein levels of complex II and IV subunits were significantly restored upon PQQ treatment ($P < 0.05$, S2 Fig).

<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0143600>

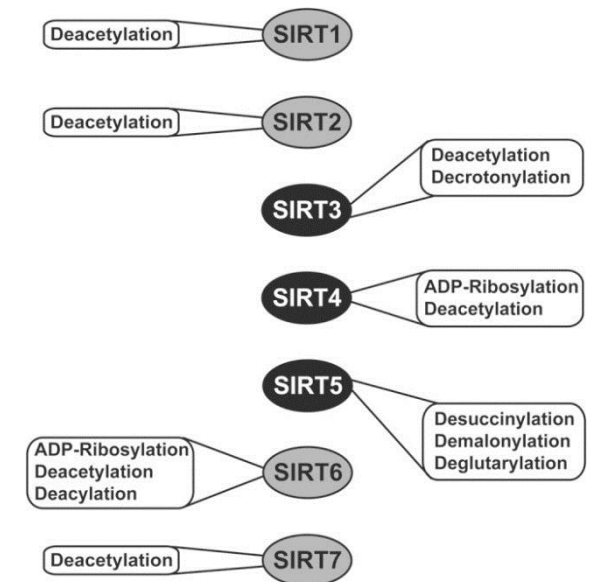
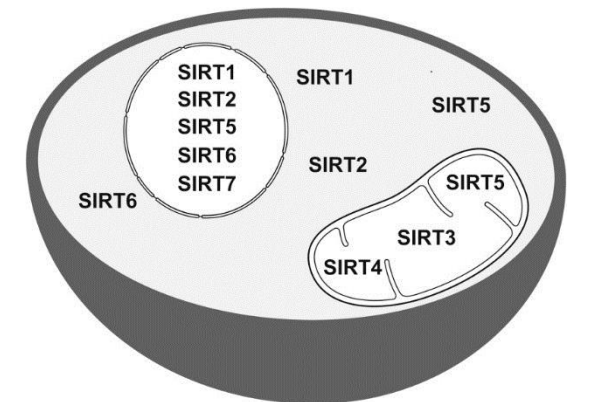
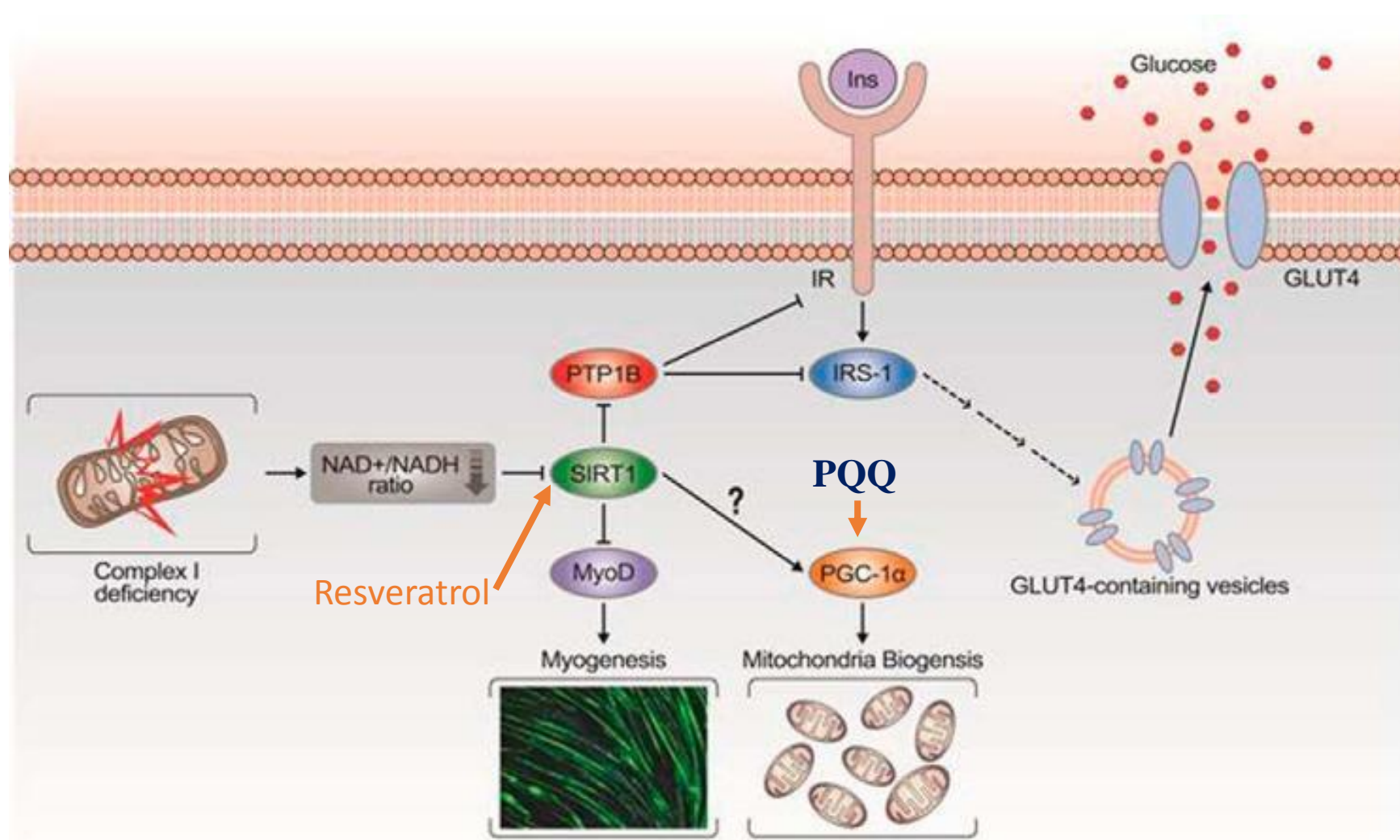
[J Biol Chem.](#) 2010 Jan 1;285(1):142-52.

Pyrroloquinoline quinone (**PQQ**) stimulates mitochondrial biogenesis through cAMP response element-binding protein (**CREB**) phosphorylation and increased **PGC-1 α** expression.



Mitochondrial biogenesis occurs through the combined effects of **genes** activated by **PQQ** via the following three mechanisms:

1. **PQQ increases** expression of **PGC-1 α** .
2. **PQQ activates** a signaling protein known as cAMP-response element-binding protein or **CREB**.
3. **PQQ regulates** a recently discovered gene called **DJ-1**. As with PGC-1 α and CREB, DJ-1 is intrinsically involved in cell function and survival.



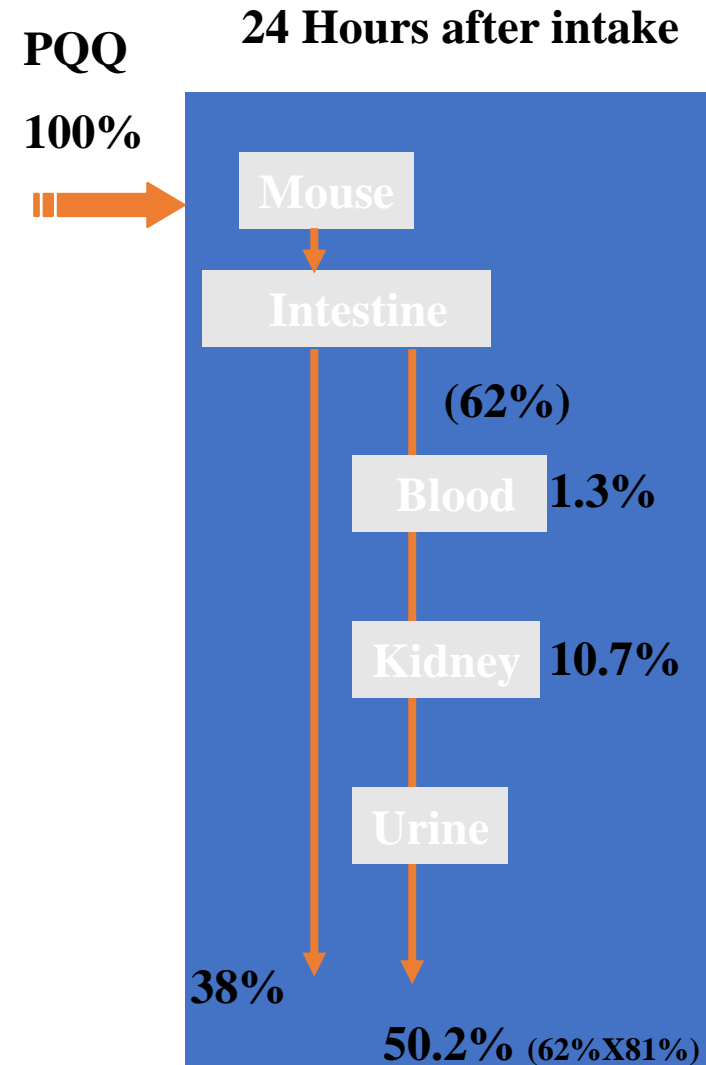
Model explaining how Complex I dysfunction induces myogenesis enhancement and insulin resistance. Complex I dysfunction decreases the NAD⁺/NADH ratio, which leads to SIRT1 inactivation. Thus, MyoD is acetylated and activated because of an inactive SIRT1 deacetylase, which enhances skeletal myogenesis. In addition, the PTP1B protein level is increased because of inactive SIRT1, which blunts insulin-elicited tyrosine phosphorylation of IR and IRS-1. However, mitochondrial biogenesis is not regulated by SIRT1 during skeletal myogenesis.

- SIRT1 stands for **sirtuin (silent mating type information regulation 2 homolog) 1**; Sirtuins act primarily by removing [acetyl](#) groups from [lysine](#) residues within proteins in the presence of [NAD⁺](#)

PQQ – Tissue and Organ distribution

<i>Tissue/Organ</i>	<i>Radioactivity</i>	
	<i>6 hours after intake</i>	<i>24 hours after intake</i>
<i>Plasma</i>	<i>0.4</i>	<i>0.1</i>
<i>Erythrocyte</i>	<i>10.6</i>	<i>1.2</i>
<i>Liver</i>	<i>5.4</i>	<i>1.5</i>
<i>Kidney</i>	<i>3.5</i>	<i>10.7</i>
<i>Spleen</i>	<i>0.3</i>	<i>0.0</i>
<i>Hearth</i>	<i>0.2</i>	<i>0.0</i>
<i>Lung</i>	<i>0.4</i>	<i>0.1</i>
<i>Brain</i>	<i>0.2</i>	<i>0.0</i>
<i>Adrenal Gland</i>	<i>0.0</i>	<i>0.0</i>
<i>Others</i>	<i>16.5</i>	<i>3.7</i>
<i>Skin</i>	<i>0.3</i>	<i>1.3</i>

Smidt CR et al. (1991)



Structure-Function Claims of PQQ

➤ A novel antioxidant

PQQ protects human body and organs against aging.

- PQQ is a small quinone molecule which has the ability to be a REDOX agent, capable of reducing oxidants (an antioxidant effect) and then being recycled by glutathione back into an active form.
- It appears to be quite stable as it can undergo **20-thousand cycles** before being used up, and it is novel since it associates with protein structures inside the cell

➤ Protects and increases the functionality of existing mitochondria, and also promotes the generation of new mitochondria (Mitochondrial Biogenesis).

Increased mitochondria = increased energy production.

➤ Stimulates production of Nerve Growth Factor (NGF)

NGF triggers growth of nerve cells to repair damaged nerves from stroke or other injury.

Figure 4
PQQ Directly Correlates with Antioxidation

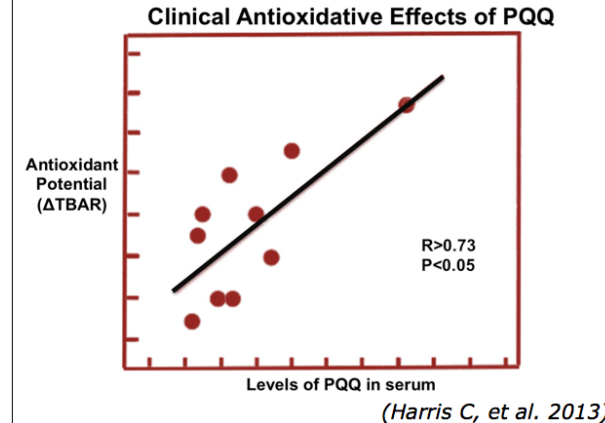


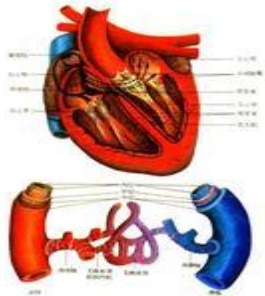
Table 3	
Compound	Catalytic redox cycling potential
PQQ	20,000
Quercetin	800
Epicatechin	700
Norepinephrine	200
Epinephrine	100
Catechin	75
DOPA	20
6-OH-DOPA	20
Ascorbic Acid	4

Benefits by Using PQQ



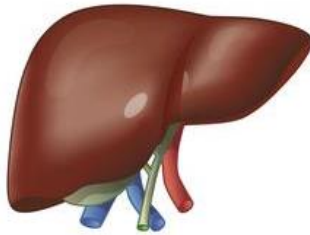
Cognitive Support

Prevents cognitive decline (memory loss, learning difficulty, etc.) due to age, stroke or neurodegenerative disorders.
Protects and restores damaged brain and nerve cells.



Cardiovascular Support

Supports energy (ATP) metabolism by the mitochondria.
Provides powerful antioxidant protection from damaging free radicals.



Liver Metabolism Support

Rescue acute and chronic liver injury caused by various factors.



Proven Benefits of PQQ (Human and In Vivo)

- **PQQ Decreases Inflammation Biomarkers and Free Radicals**
 - Healthy humans who took 20mg of [PQQ](#) resulted in significant decreases chronic inflammation biomarkers of C-reactive protein (by 45% after 3 weeks) and [IL-6](#).
- **PQQ Creates New Mitochondria and Enhance Mitochondrial Function**
 - Stimulate mitochondrial biogenesis are linked to many health benefits such increased longevity, improved energy utilization, and protection from free radicals.
 - By increasing cellular metabolism it favorably affects blood pressure, cholesterol and triglyceride breakdown, and the onset of [obesity](#) .
 - Mice and rats fed diets lacking in pyrroloquinoline quinone ([PQQ](#)) have reduced mitochondrial content.
- **PQQ Improves Memory and Reasoning**
 - A study with middle ages and elderly people found [PQQ](#) + CoQ10 led to a significant increase in performance in the Stroop test (measures reasoning) and reaction tests.
 - PQQ Improves Brain Function By Increasing Nerve Growth Factor and Schwann Cells
 - PQQ is Neuroprotective Against Alzheimer's, Parkinson's and Cognitive Injuries
- **PQQ Improves Sleep, Mood, and Fatigue**
 - One open-label human study conducted with 20mg PQQ for 8 weeks in 17 persons with fatigue or sleep-impairing disorder noted that PQQ was able to significantly improve sleep quality, with improvements in sleep duration and quality appearing at the first testing period after 4 weeks. It also led to a decrease in the time it took to fall asleep but required 8 weeks to reach significance.
- **PQQ Protects Brain and Heart Against Stroke**
 - PQQ administration reduces the size of damaged areas in animal models of acute brain stroke and heart attack.
- **PQQ Decreases Insulin Resistance**
 - PQQ alleviates fat-induced [insulin resistance](#) by increasing mitochondrial biogenesis in muscle cells, similar to exercise
- **PQQ Slows Down the Progression of Osteoarthritis by Inhibiting Nitric Oxide Production and Metalloproteinase Synthesis**
- **PQQ Improved Dry Skin Condition in Women**

PQQ is associated with mitochondrial functions and biogenesis (quantities)

MITOCHONDRIA

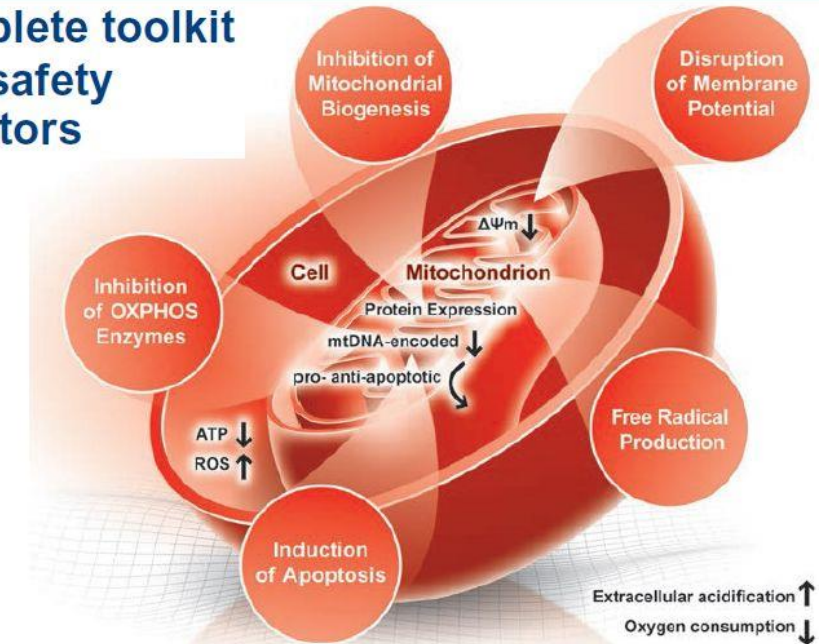
- Organelles that produce energy from food
- AKA the powerhouse b/c they release energy from food
- Some muscle cells have 20,000 mitochondria
- Found in both plant and animal cells



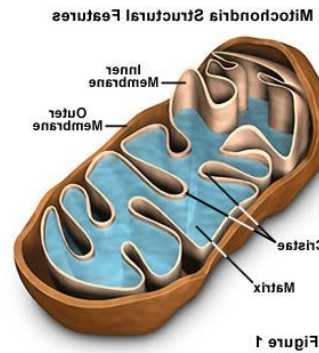
MitoTox™ Mitochondrial toxicity application guide

abcam®
discover more

The complete toolkit
for drug safety
investigators



Mitochondria



- Mitochondria are rod-shaped organelles that can be considered the power generators of the cell, converting oxygen and nutrients into adenosine triphosphate (ATP).
 - ATP is the chemical energy "currency" of the cell that powers the cell's metabolic activities.
 - This process is called aerobic respiration and is the reason animals breathe oxygen.
 - Without mitochondria (singular, mitochondrion), higher animals would likely not exist because their cells would only be able to obtain energy from anaerobic respiration (in the absence of oxygen), a process much less efficient than aerobic respiration.
 - In fact, mitochondria enable cells to produce 15 times more ATP than they could otherwise, and complex animals, like humans, need large amounts of energy in order to survive.
- Typically, a sperm carries mitochondria in its tail as an energy source for its long journey to the egg. When the sperm attaches to the egg during fertilization, the tail falls off. Consequently, the only mitochondria the new organism usually gets are from the egg its mother provided.
- Mitochondria are similar to plant chloroplasts, which work in a different manner to convert energy from the sun into the biosynthesis of required organic nutrients using carbon dioxide and water.

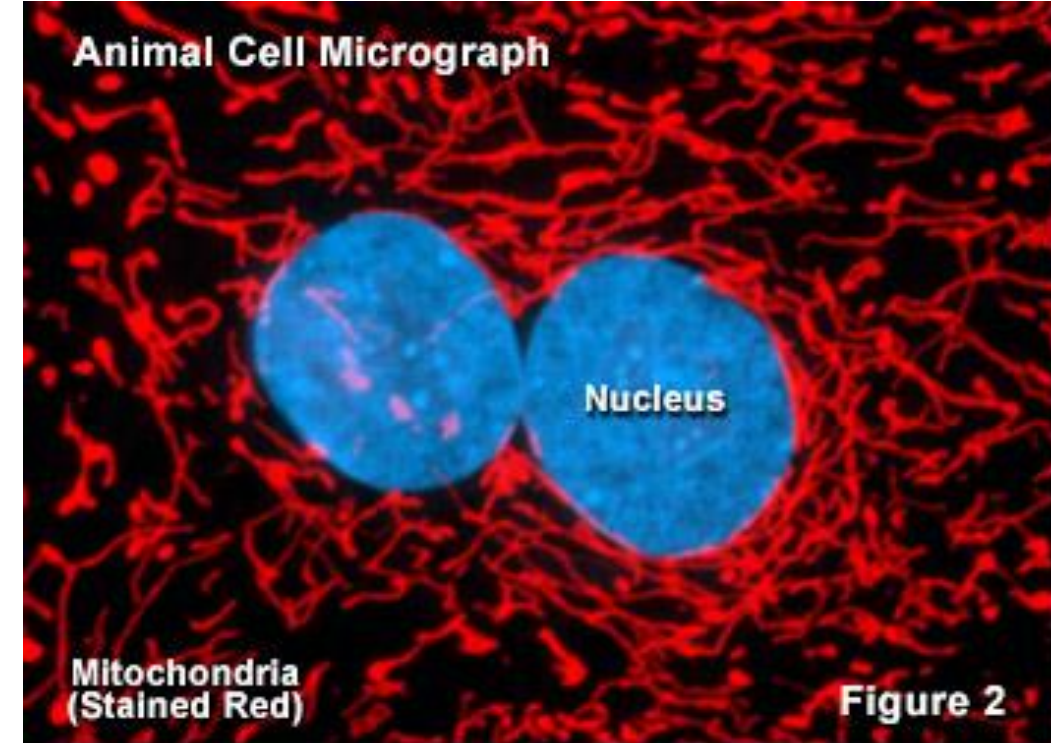


Figure 2

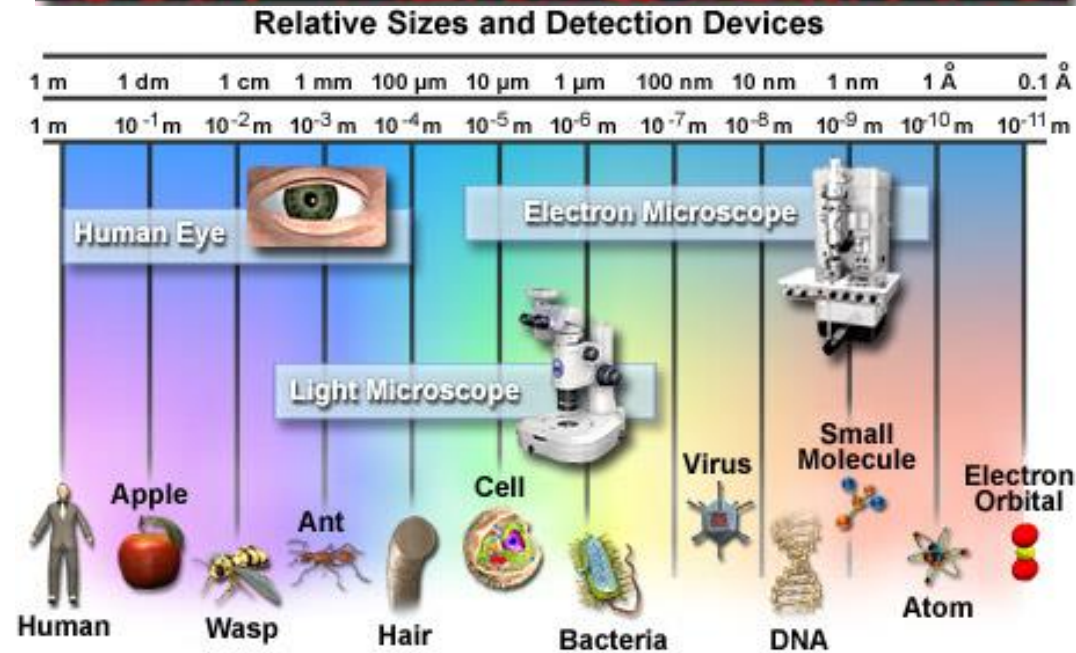


Figure 1

Energy Factories and Much More

- The conventional teaching in biology and medicine is that mitochondria function only as “energy factories” for the cell. This over-simplification is a mistake which has slowed our progress toward understanding the biology underlying mitochondrial disease.
- It takes about 3000 genes to make a mitochondrion.
 - Mitochondrial DNA encodes just 37 of these genes; the remaining genes are encoded in the cell nucleus and the resultant proteins are transported to the mitochondria.
 - Only about 3% of the genes necessary to make a mitochondrion (100 of the 3000) are allocated for making ATP.
 - More than 95% (2900 of 3000) are involved with other functions tied to the specialized duties of the differentiated cell in which it resides.
- These non-ATP-related functions are intimately involved with most of the major metabolic pathways used by a cell to build, break down, and recycle its molecular building blocks.
 - Cells cannot even make the RNA and DNA they need to grow and function without mitochondria.
 - The building blocks of RNA and DNA are purines and pyrimidines.
 - Mitochondria contain the rate-limiting enzymes for pyrimidine biosynthesis (dihydroorotate dehydrogenase) and heme synthesis (d-amino levulinic acid synthetase) required to make hemoglobin.
- In the liver, mitochondria are specialized to detoxify ammonia in the urea cycle. Mitochondria are also required for cholesterol metabolism, for estrogen and testosterone synthesis, for neurotransmitter metabolism, and for free radical production and detoxification.

Nutrients Helpful for Mitochondria Diseases

First Tier Supplements

<i>Supplement</i>	<i>Dose Range</i>
CoQ10	5 – 15 mg/kg/day
Levo-carnitine (Carnitor)	Variable, starting dose of 30 mg/kg/day, typical maximum of 100 mg/kg/day
Riboflavin (B2)	100 – 400 mg a day

Second Tier Supplement

<i>Supplement</i>	<i>Dose Range</i>
Acetyl-L-Carnitine	250 – 1000 mg per day
Thiamine (B1)	50 – 100 mg a day
Niacin (B3)	50 – 100 mg a day
Vitamin E	200 – 400 IU; 1 – 3 times a day
Vitamin C	100 – 500 mg; 1 – 3 times a day
Lipoic Acid (a -lipoate)	60 – 200 mg; 3 times a day
Selenium	25 – 50 micrograms a day
b -carotene	10,000 IU; every other day to daily
Biotin	2.5 – 10 mg a day
Folic Acid	1 – 10 mg a day

Nutrients Helpful for Mitochondria Diseases

- **PQQ**
- **Coenzyme Q₁₀** is a key cofactor and component of the mitochondrial electron transport chain.
 - the main role of CoQ₁₀ is its involvement in the transfer of electrons along the multiple complexes of the mitochondrial electron transport chain.
- **Alpha-lipoic acid** acts as a critical cofactor in mitochondrial α -keto acid dehydrogenases, and thus it is important in mitochondrial, oxidative-decarboxylation reactions.
- **L-carnitine** is directly involved in the transport of fatty acids into the mitochondrial matrix for subsequent β -oxidation, but it also functions in removal of excess acyl groups from the body and in the modulation of intracellular coenzyme A (CoA) homeostasis.
 - L-carnitine deficiency disorders are associated with reduced mitochondrial function, insulin resistance, and coronary artery disease
- **NADH** functions as a cellular redox cofactor in over 200 cellular redox reactions and as substrate for certain enzymes. In the mitochondria, NADH delivers electrons from metabolite hydrolysis to the electron transport chain, but in its reduced form, it can also act as a strong antioxidant.
 - Its deficiency results in pellagra, which is characterized by dermatitis, diarrhea, dementia, and eventually death.
 - The usual route of dietary supplementation has historically been via NADH precursors, such as niacin, nicotinic acid, or nicotinamide, but recently, microcarriers have been used to stabilize oral NADH so that it can be directly ingested in small doses and absorbed in the gastrointestinal system.
- **Phospholipids:** Oral membrane phospholipids can increase mitochondrial function and decrease fatigue in chronic fatigue syndrome, fibromyalgia syndrome, and other fatiguing conditions, including natural aging.
 - Oral administration of NT Factor (for 12 weeks resulted in a 35.5% reduction in fatigue and 26.8% increase in mitochondrial function
 - membrane phospholipids (2000 mg/d), CoQ₁₀ (35 mg/d), microencapsulated NADH (35 mg/d), L-carnitine (160 mg/d), α -ketoglutaric acid (180 mg/d), into an oral supplement (ATP Fuel) can help restore mitochondrial function and reduce intractable fatigue in patients with chronic illnesses

Mitochondrial Dysfunction

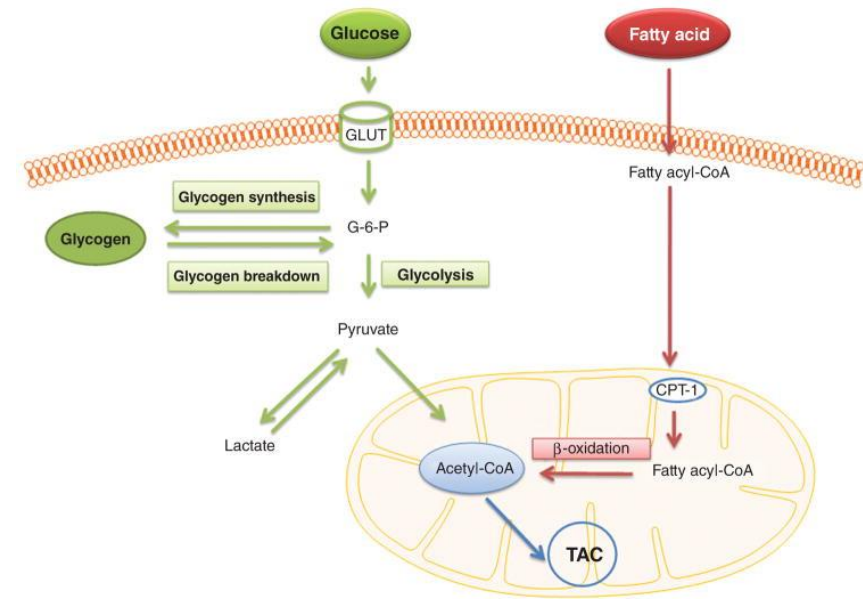
Loss of Oxidative-Phosphorylation with subsequent declines in ATP generation

Impaired Mitochondrial Activity in the Insulin-Resistant Offspring of Patients with Type 2 Diabetes **Petersen et.al. PLoS 2005**

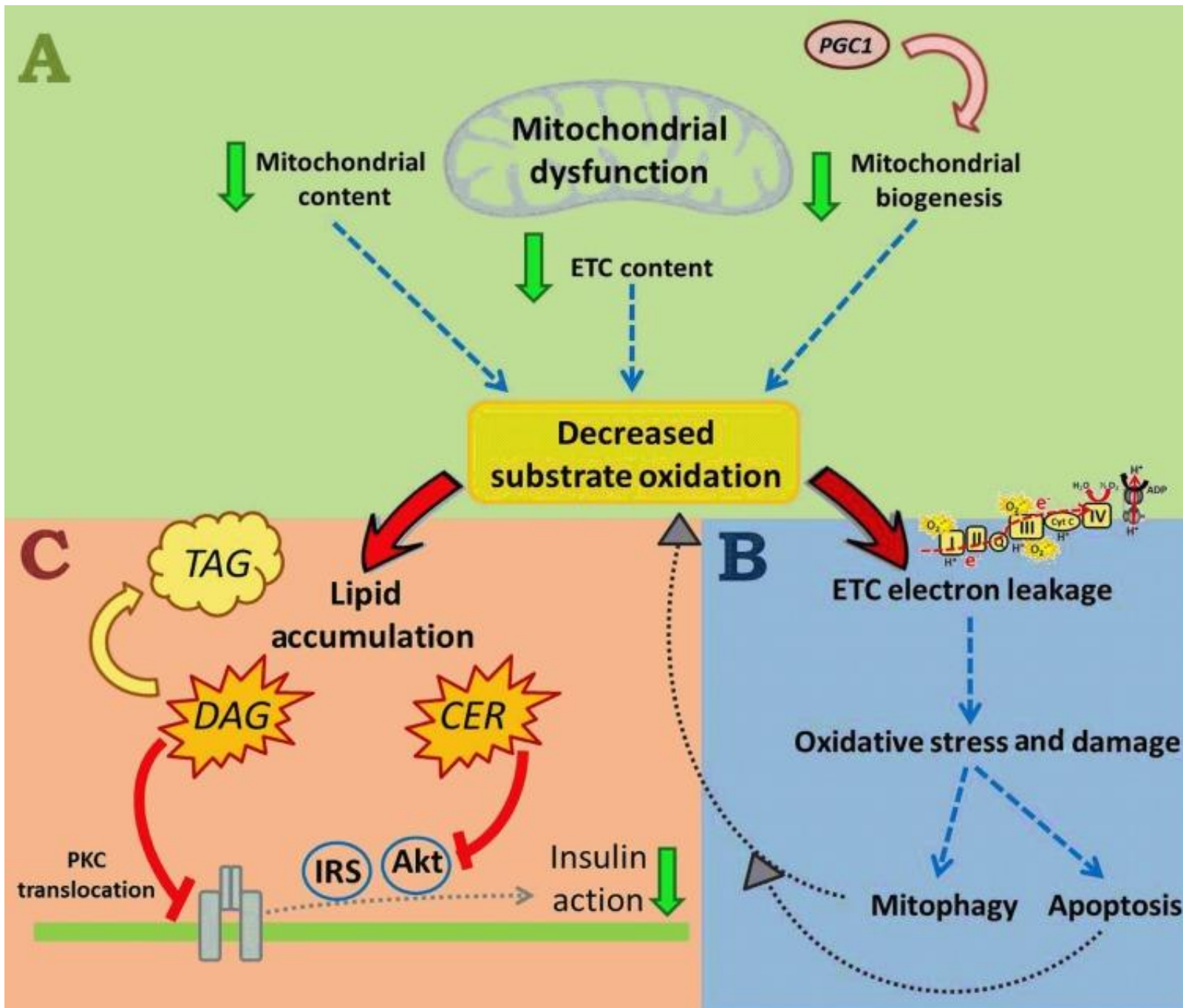
MRS (magnetic resonance spectroscopy) assessment of ATP-Synthase flux and intramyocellular inorganic phosphate in healthy, normoglycemic (i.e. not “diabetic”) lean Insulin-resistant offspring (n=13) of T2DM patients **versus** non-insulin resistant health lean controls (n=10)

Results:

- 1) Insulin stimulated Glucose uptake declined **50%** in IR group
- 2) Rates of mitochondrial phosphorylation in skeletal muscle were **30%** lower in IR group.
- 3) 2-Fold increase in **Intra Myocellular Lipid (IML)** Content in IR Group
- 4) is most likely attributable to **acquired defects in mitochondrial biogenesis**, which lead to reductions in skeletal-muscle mitochondrial content as well as function



G-6-P, glucose 6-phosphorylase;
CPT-1, carnitine palmitoyl transferase I;
TAC, tricarboxylic acid cycle.



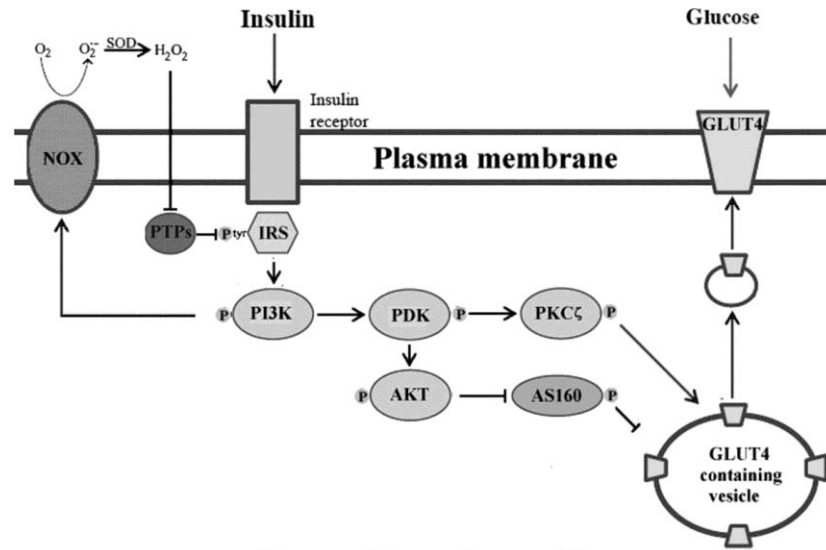
Mitochondrial dysfunction includes a **reduction** in mitochondrial **content** and mitochondrial **biogenesis**, and/or a decrease in the expression of mitochondrial **oxidative proteins**, such as complexes of the electron transport chain (ETC), with all those changes likely leading to decreased substrate oxidation (A).

A diminished electron flow through the ETC can subsequently cause electron leakage and superoxide generation, followed by oxidative stress and damage. In a healthy environment, mitochondria can respond to damage through **mitophagy** pathways (removal of damaged mitochondria, preventing cell death), or in the case of high cellular stress, with **apoptosis** (B),

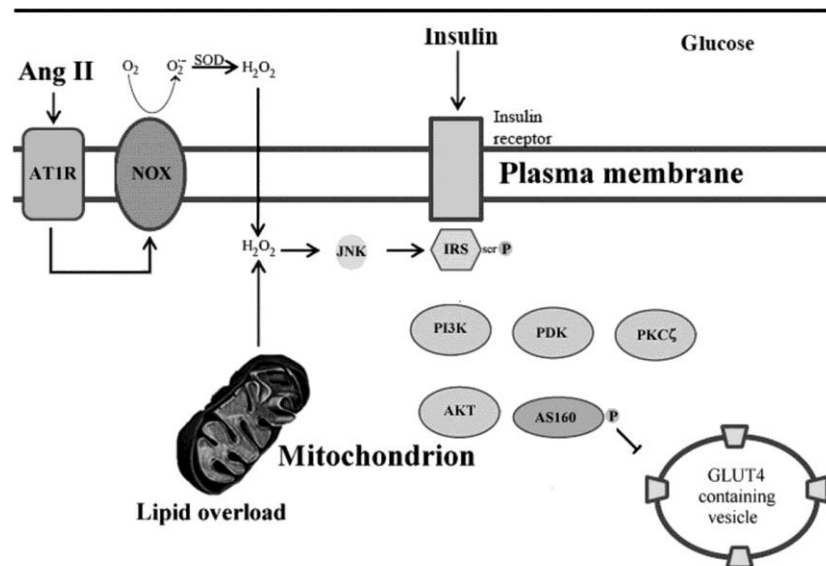
both aggravating the decrease in substrate utilisation, and all up leading to increased lipid accumulation (C).

Active lipid intermediates, such as diacylglycerols (**DAG**) and ceramide (**CER**) then cause **inhibition of the insulin signalling** pathway.

Insulin-stimulated glucose uptake in skeletal muscle and its inhibition in obese or high-fat diet-fed animals.

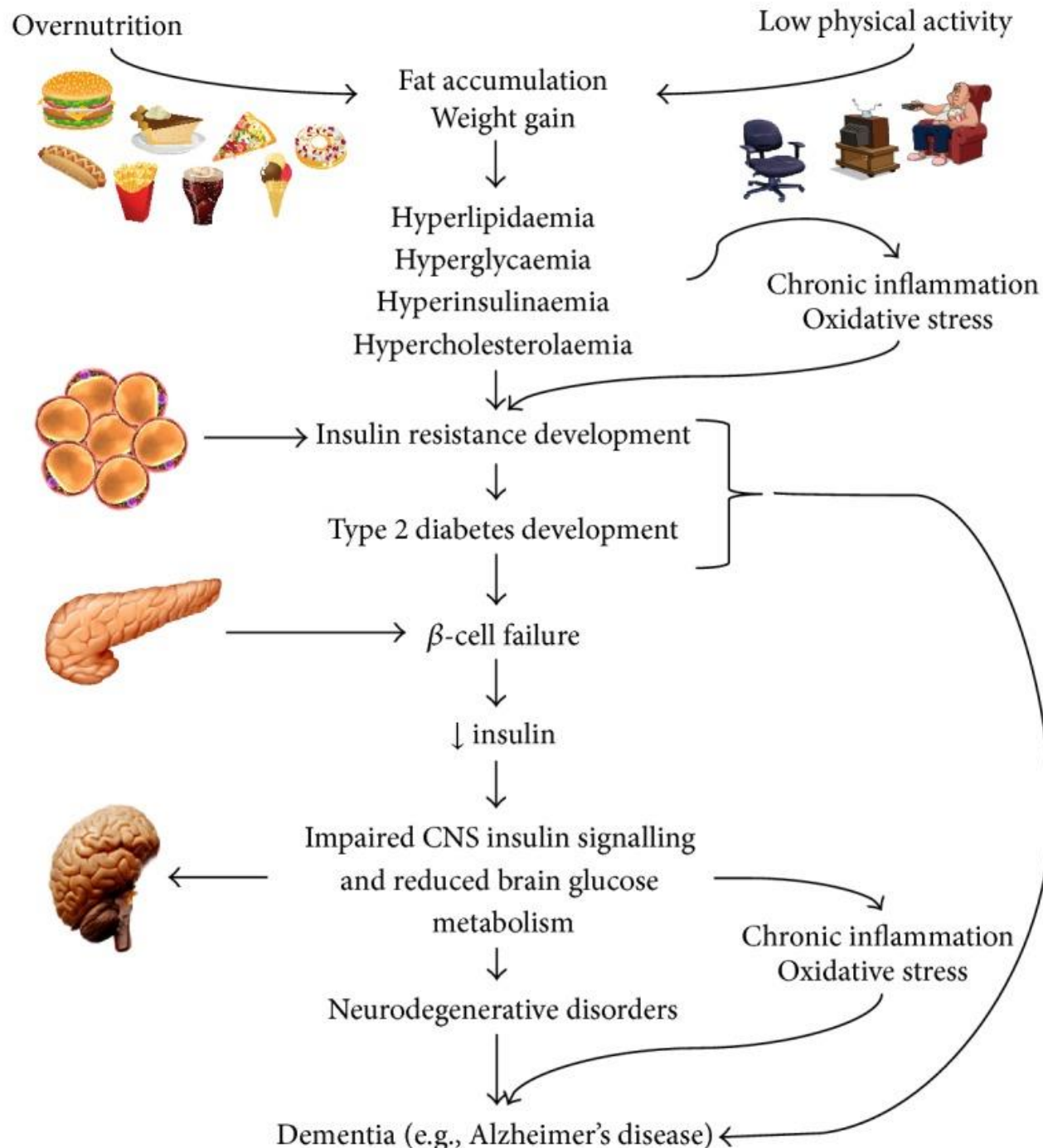


Insulin signaling



Insulin resistance

GLUT4, glucose transporter type 4;
NOX, NADPH oxidase;
SOD, superoxide dismutase;
Ang II, angiotensin II;
AT1R, Ang II type 1 receptor;
IRS, insulin receptor substrate;
PI3K, phosphatidylinositol 3-kinase;
PTPs, protein tyrosine phosphatases;
PKCζ, atypical protein kinase C;
PDK, phosphoinositide-dependent kinase;
Akt, protein kinase B;
AS160, 160 kDa protein;
JNK, c-Jun amino-terminal kinases.



Neurodegeneration, insulin resistance, obesity, and T2DM.

Mitochondrial metabolism (primary metabolic target of PGC-1 α) disturbances are widely acknowledged contributors to type 2 diabetes development.

Metabolic overload, chronic inflammation, and oxidative stress promote cellular dysregulation in both T2DM and AD.

Brain IR may occur in the absence of diabetes suggesting that AD may develop in the earlier stages of insulin resistance.

Chronic inflammation and oxidative stress are considered two key factors linking diabetes and AD

[FASEB J.](#) 2017 Apr;31(4):1434-1448.

Early PQQ supplementation has persistent long-term protective effects on developmental programming of hepatic lipotoxicity and inflammation in obese mice.

Nonalcoholic fatty liver disease (NAFLD) is widespread in adults and children. Early exposure to maternal obesity or Western-style diet (WD) increases steatosis and oxidative stress in fetal liver and is associated with lifetime disease risk in the offspring.

Pyrroloquinoline quinone (PQQ) is a natural antioxidant **found in soil, enriched in human breast milk, and essential for development in mammals**. We investigated whether a supplemental dose of PQQ, provided prenatally in a mouse model of diet-induced obesity during pregnancy, could **protect obese offspring** from progression of **NAFLD**. PQQ treatment given **pre- and postnatally** in WD-fed offspring had **no effect on weight gain** but **increased metabolic flexibility while reducing body fat and liver lipids**, compared with untreated obese offspring. Indices of NAFLD, including **hepatic ceramide levels**, oxidative stress, and expression of proinflammatory genes (*Nos2*, *Nlrp3*, *Il6*, and *Ptgs2*), were decreased in WD PQQ-fed mice, concomitant with increased expression of fatty acid oxidation genes and decreased *Pparg* expression. Notably, these changes **persisted even after PQQ withdrawal** at weaning. Our results suggest that supplementation with **PQQ, particularly during pregnancy and lactation**, protects offspring from WD-induced developmental programming of hepatic lipotoxicity and may help slow the advancing epidemic of NAFLD in the next generation.

Calorie restriction increases muscle mitochondrial biogenesis in healthy humans.

BACKGROUND: Caloric restriction without malnutrition extends life span in a range of organisms including insects and mammals and lowers free radical production by the mitochondria. However, the mechanism responsible for this adaptation are poorly understood.

METHODS AND FINDINGS: The current study was undertaken to examine muscle mitochondrial bioenergetics in response to caloric restriction alone or in combination with exercise in 36 young (36.8 ± 1.0 y), overweight (body mass index, 27.8 ± 0.7 kg/m²) individuals randomized into one of three groups for a 6-mo intervention: Control, 100% of energy requirements; CR, 25% caloric restriction; and CREX, caloric restriction with exercise (CREX), 12.5% CR + 12.5% increased energy expenditure (EE). In the controls, 24-h EE was unchanged, but in CR and CREX it was significantly reduced from baseline even after adjustment for the loss of metabolic mass (CR, -135 ± 42 kcal/d, $p = 0.002$ and CREX, -117 ± 52 kcal/d, $p = 0.008$). Participants in the CR and CREX groups had increased expression of genes encoding proteins involved in mitochondrial function such as PPARGC1A, TFAM, eNOS, SIRT1, and PARL (all, $p < 0.05$). In parallel, mitochondrial DNA content increased by $35\% \pm 5\%$ in the CR group ($p = 0.005$) and $21\% \pm 4\%$ in the CREX group ($p < 0.004$), with no change in the control group ($2\% \pm 2\%$). However, the activity of key mitochondrial enzymes of the TCA (tricarboxylic acid) cycle (citrate synthase), beta-oxidation (beta-hydroxyacyl-CoA dehydrogenase), and electron transport chain (cytochrome C oxidase II) was unchanged. DNA damage was reduced from baseline in the CR (-0.56 ± 0.11 arbitrary units, $p = 0.003$) and CREX (-0.45 ± 0.12 arbitrary units, $p = 0.011$), but not in the controls. In primary cultures of human myotubes, a nitric oxide donor (mimicking eNOS signaling) induced mitochondrial biogenesis but failed to induce SIRT1 protein expression, suggesting that additional factors may regulate SIRT1 content during CR.

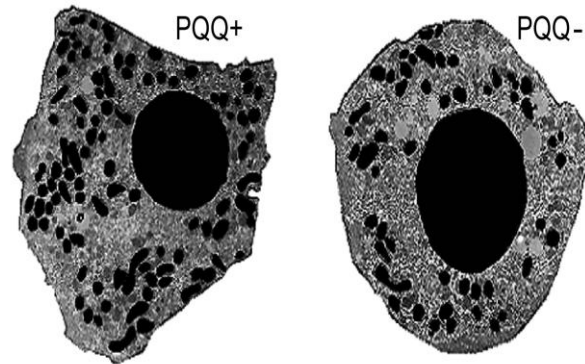
CONCLUSIONS: The observed increase in muscle mitochondrial DNA in association with a decrease in whole body oxygen consumption and DNA damage suggests that caloric restriction improves mitochondrial function in young non-obese adults.

PQQ stimulates mitochondrial biogenesis

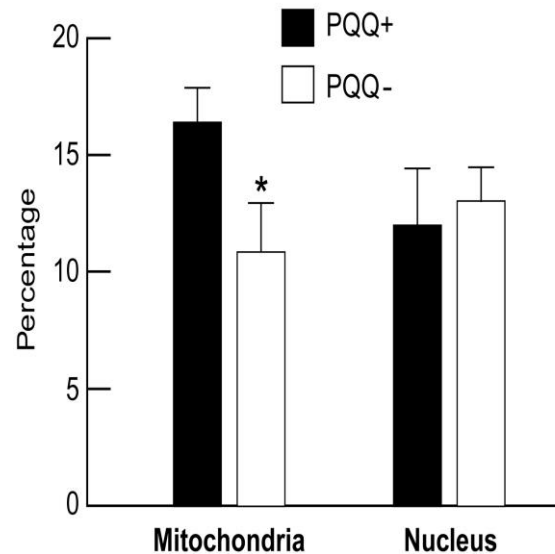
- Bioactive compounds reported to stimulate mitochondrial biogenesis are linked to many health benefits such increased longevity, improved energy utilization, and protection from reactive oxygen species.
- Studies have shown that mice and rats fed diets lacking in pyrroloquinoline quinone (PQQ) have reduced mitochondrial content.
- Exposure of mouse Hepa1-6 cells to 10-30 microm PQQ for 24-48 h resulted in increased citrate synthase and cytochrome C oxidase activity, Mitotracker staining, mitochondrial DNA content, and cellular oxygen respiration.
- PQQ exposure stimulated phosphorylation of CREB at serine 133, activated the promoter of PGC-1alpha, and increased PGC-1alpha mRNA and protein expression.
- Consistent with activation of the PGC-1alpha pathway, PQQ increased nuclear respiratory factor activation (NRF-1 and NRF-2) and Tfam, TFB1M, and TFB2M mRNA expression.
- The ability of PQQ to stimulate mitochondrial biogenesis accounts in part for action of this compound and suggests that PQQ may be beneficial in diseases associated with mitochondrial dysfunction.

Mitochondrial Biogenesis

PQQ dietary status influences mitochondrial content in BALB/c mice



△ The small darkened areas correspond to mitochondrial cross-sectional areas

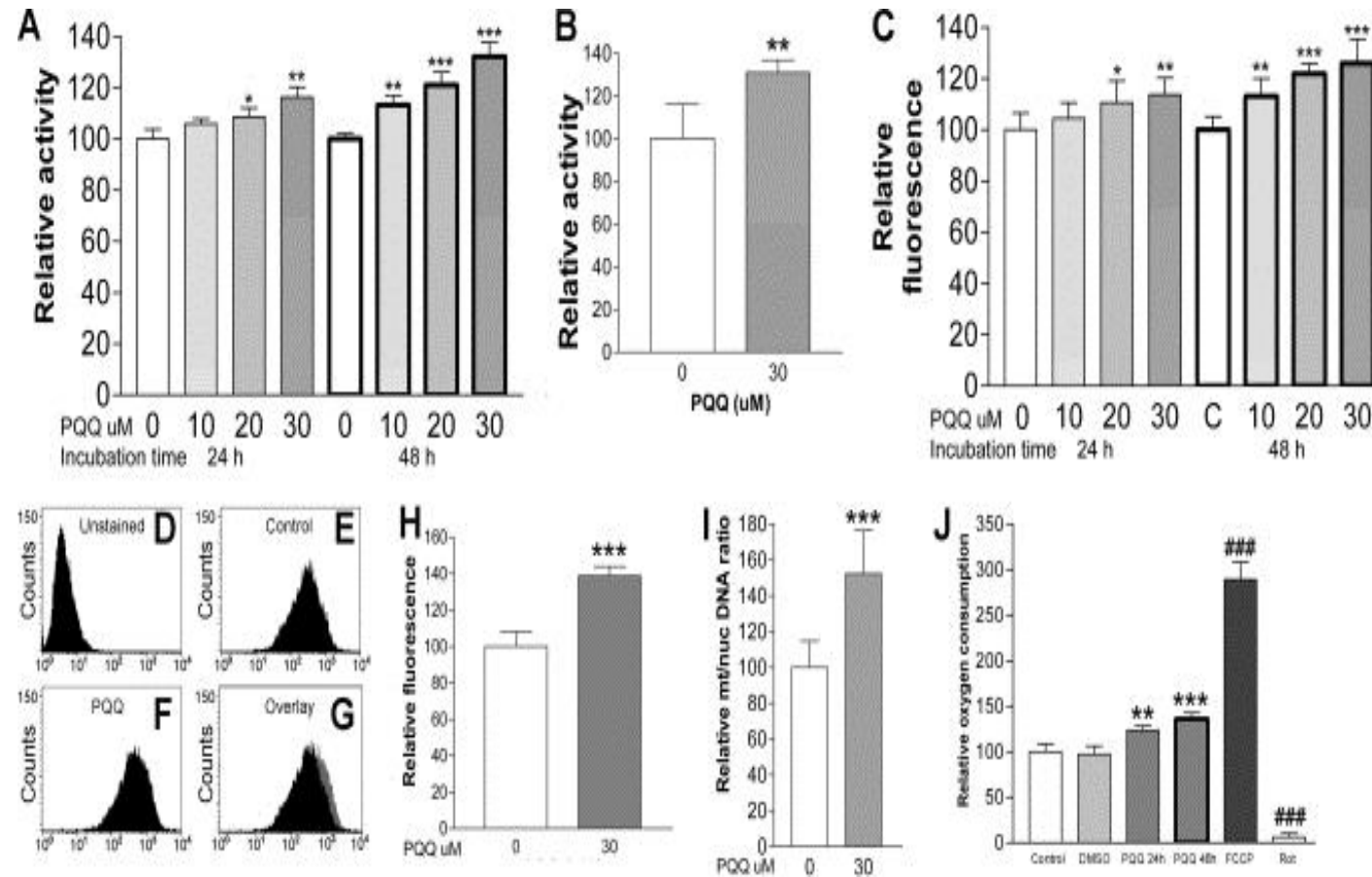


Mitochondrial content and the respiratory control ratio for liver from PQQ-deficient and -supplemented BALB/c mice¹

Item	PQQ+	PQQ -
RCR	2.44 ± 0.12	2.16 ± 0.24
Functional mitochondrial preparations, %	78	29*
Mitochondria, <i>n</i> /cell	91.0 ± 6.6	56.8 ± 7.8*
Size of individual mitochondria, μm^2	0.823 ± 0.070	0.808 ± 0.081

¹ Values are means \pm SEM or %, *n* = 6. *Different from PQQ+, *P* < 0.01.

PQQ induces mitochondrial biogenesis in Hepa1-C6 cells



A), cytochrome *c* oxidase assay

B), Mitotracker staining microplate assay

C), Mitotracker flow cytometry assay

D–H), mitochondrial to nuclear DNA ratios

I), and cellular oxygen consumption

J). Citrate synthase activity at 24 or 48 h was determined by MTT reduction assay and expressed relative to respective control conditions (without PQQ at 24 or 48 h).

Rats fed a diet deficient in PQQ are metabolically challenged, due to decreased mitochondria number

Table 1. Plasma PQQ and mtDNA/nuclear DNA Ratio Levels.

PQQ (nM) in Plasma and Tissues				
Parameters		PQQ Treatments ^{1,2}		
Experiment Designation	Tissue	PQQ+	PQQ–	PQQ–/+
Lipid Assessment	Plasma (Adult)	5.2±1.3	3.5±2.1*	17.0±4.5**
	Liver (Adult)	21.3±16.4	5.4±2.6**	26.1±17.2
	Heart (Adult)	3.4±1.9	2.4±1.0*	13.6±10.2*
Energy, Glucose, insulin, FFA Assessment	Plasma (Weanling/young)	10.1±4.7	0.71±0.35***	16.4±2.9
Glucose, insulin, FFA	Plasma (Adult)	11.3±6.7	2.6±1.35**	18.5±4.9
Ischemia Reperfusion	Plasma (Adult)	10.7±2.1	0.7±1.5**	ND
mtDNA/NuclearDNA Ratio ³				
Lipid Assessment	Liver (Adult)	1.0±0.18	0.78±0.12**	1.3±0.06
Energy, Glucose, insulin, FFA Assessment	Liver (Weanling/young)	1.0±0.18	0.84±0.07	1.06±0.08
Glucose, insulin, FFA	Liver (Adult)	1.06±0.19	0.77±0.15*	1.28±0.19
	Heart (Adult)	1.0±0.21	0.85±0.17	1.1±0.2
Ischemia Reperfusion	Liver (Adult)	1.0±0.08	0.72±0.1**	1.1±0.1

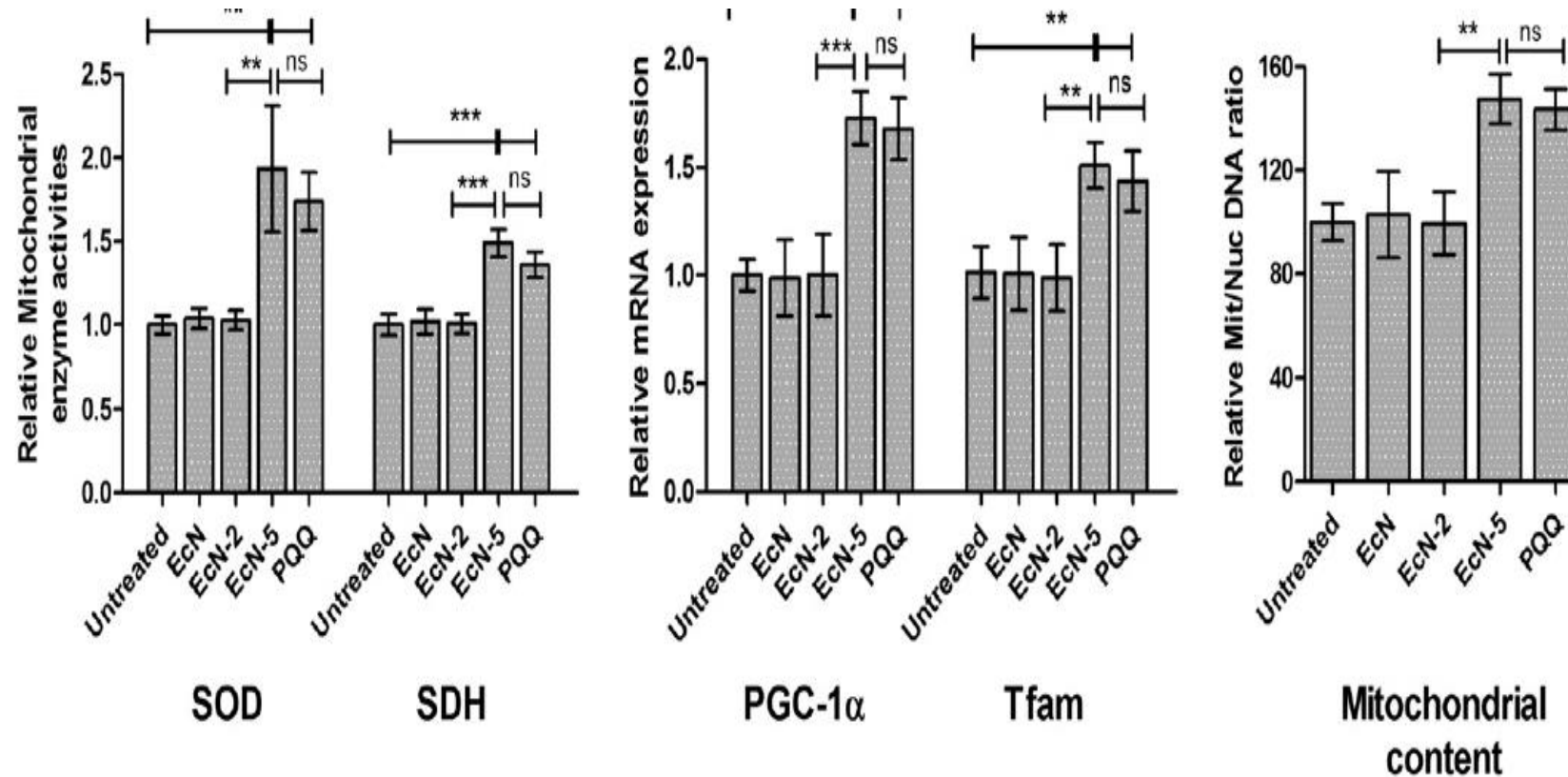
¹Rats were fed an amino acid-based semi-purified diet either deficient in PQQ (PQQ–) or with PQQ added at 2 mg/kg diet (PQQ+). Rats initially fed PQQ– diets were also repleted with PQQ by i.p. injection (4.5 mg PQQ/Kg BW/24 hours X 3).

²The superscripts *, **, *** represent significance relative to the PQQ+ group at p<0.2; <0.05; or p<0.01, respectively.

³The relative amounts of liver mitochondrial DNA (mtDNA) and nuclear DNA were measured by real-time PCR. The targeted genes were the nuclear cystic fibrosis and the mitochondrial nicotinamide adenine dinucleotide dehydrogenase-5 gene. When corresponding values for liver in each experiment are averaged, the liver values were significant at p<0.01 (PQQ+ vs PQQ– and PQQ+ vs PQQ –/+ based on ANOVA analysis using a Bonferroni correction); for heart, p<0.3.

doi:10.1371/journal.pone.0021779.t001

PQQ improves mitochondrial function in ageing rats

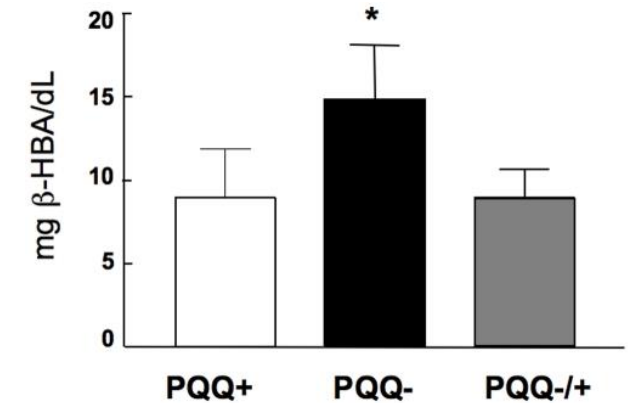
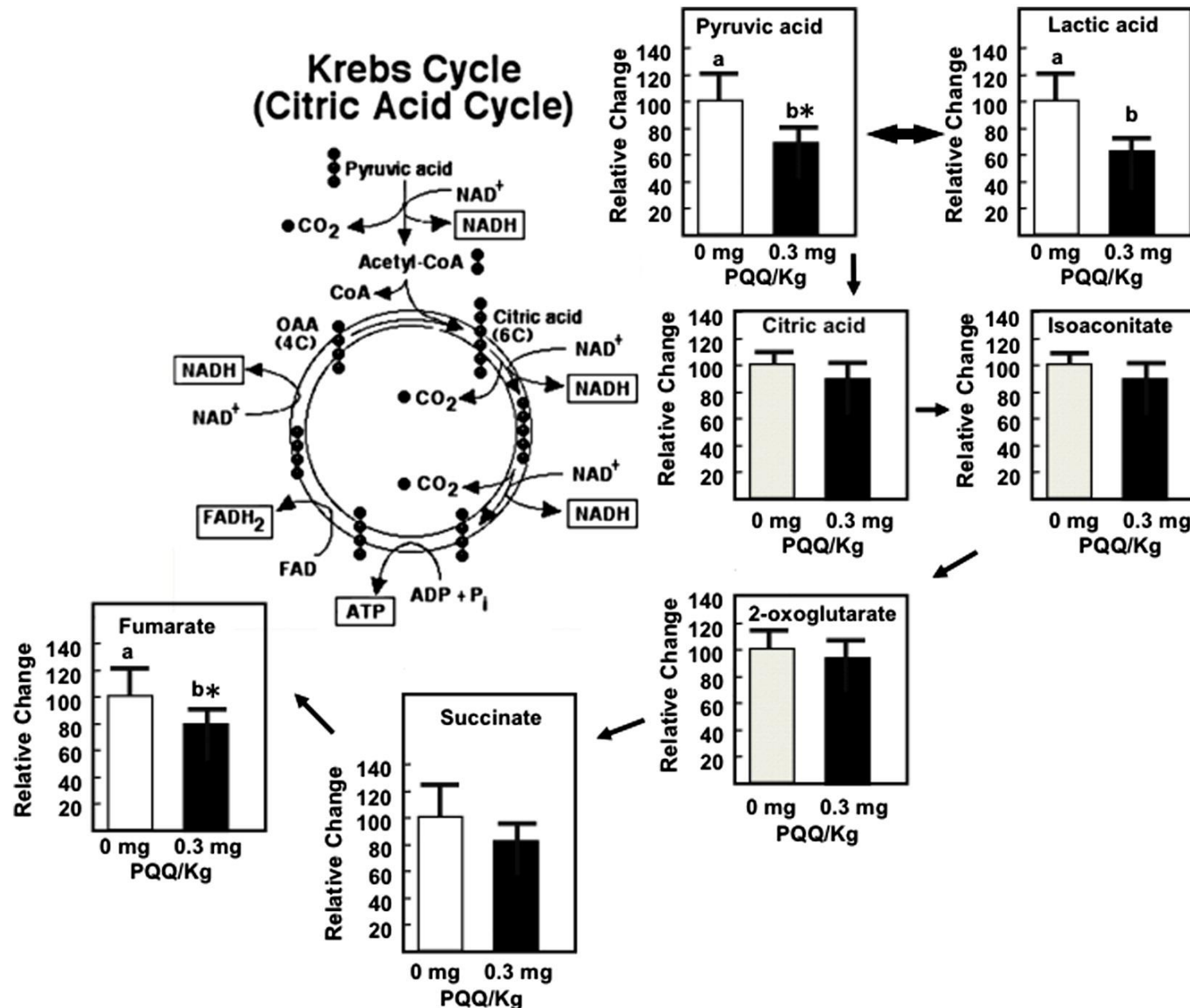


8 months feeding assay

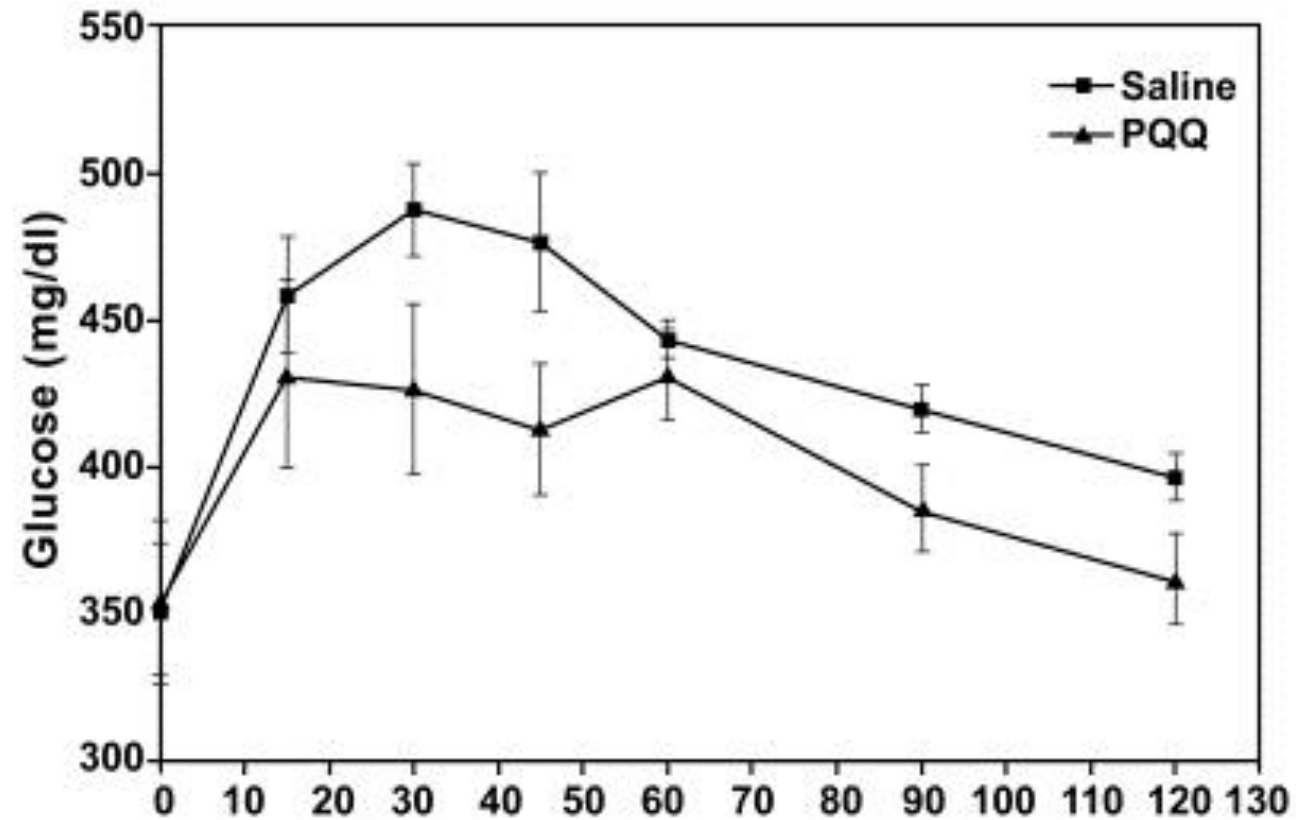
PQQ increase in mitochondrial efficiency in Human

- When humans supplement PQQ (0.075-0.3mg/kg for one week once daily),
 - urinary lactate decreased by 15% along with a reduction in urinary pyruvic acid.
 - A minor reduction of fumarate was noted, but other Krebs cycle intermediates (Isoaconitate, Citric acid, 2-oxoglutarate, and succinate) were not altered in the urine.
 - A nonsignificant decreasing trend in urinary 4-hydroxyphenylacetate was noted with PQQ; decreases in this and other urinary metabolites tend to suggest increased β -oxidation rates.
- It was hypothesized, on the assumption that urinary metabolites reflect cellular energy status, that this indicated an increase in mitochondrial efficiency.

PQQ and relative changes in urinary TCA cycle metabolites

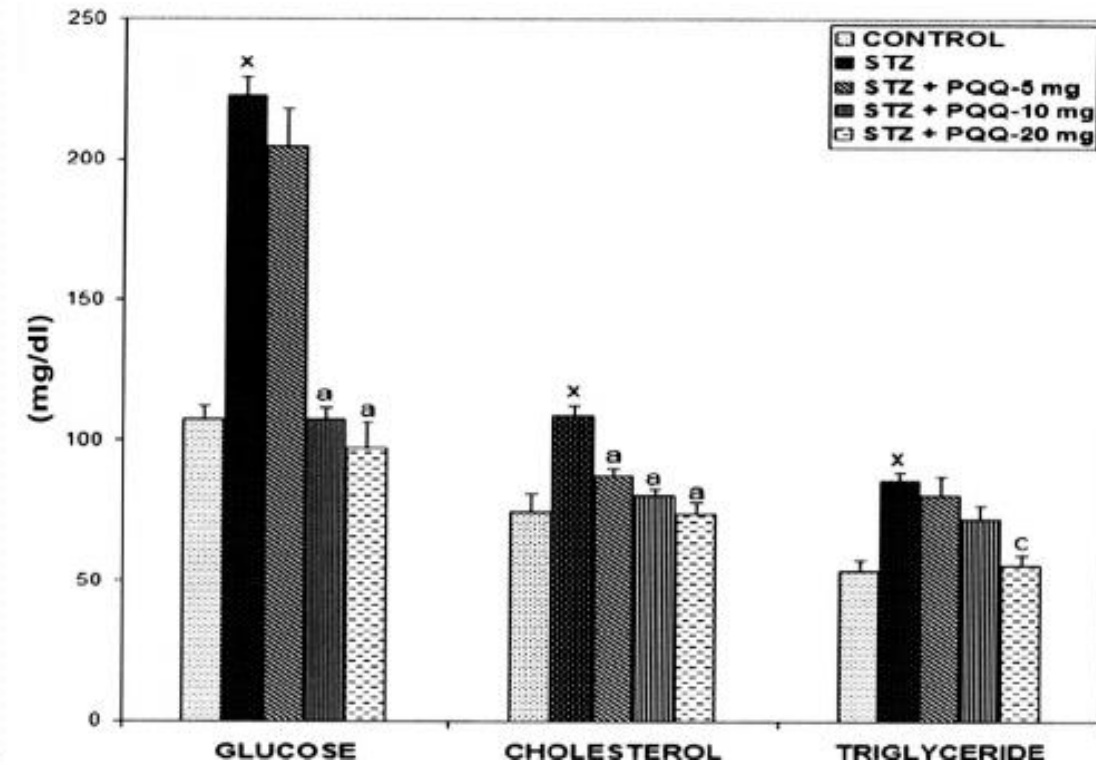


β -hydroxybutyric acid levels in rats fed the PQQ– or PQQ+ diets.
The increase in β - hydroxybutyric acid was reversed upon PQQ repletion (p<0.05). [PLoS One. 2011; 6\(7\): e21779.](#)



Oral glucose tolerance in response to a glucose load in diabetic UCD-T2DM Rats following the administration of PQQ (i.p.) at 4.5 mg PQQ/Kg BW for 3 days or saline.

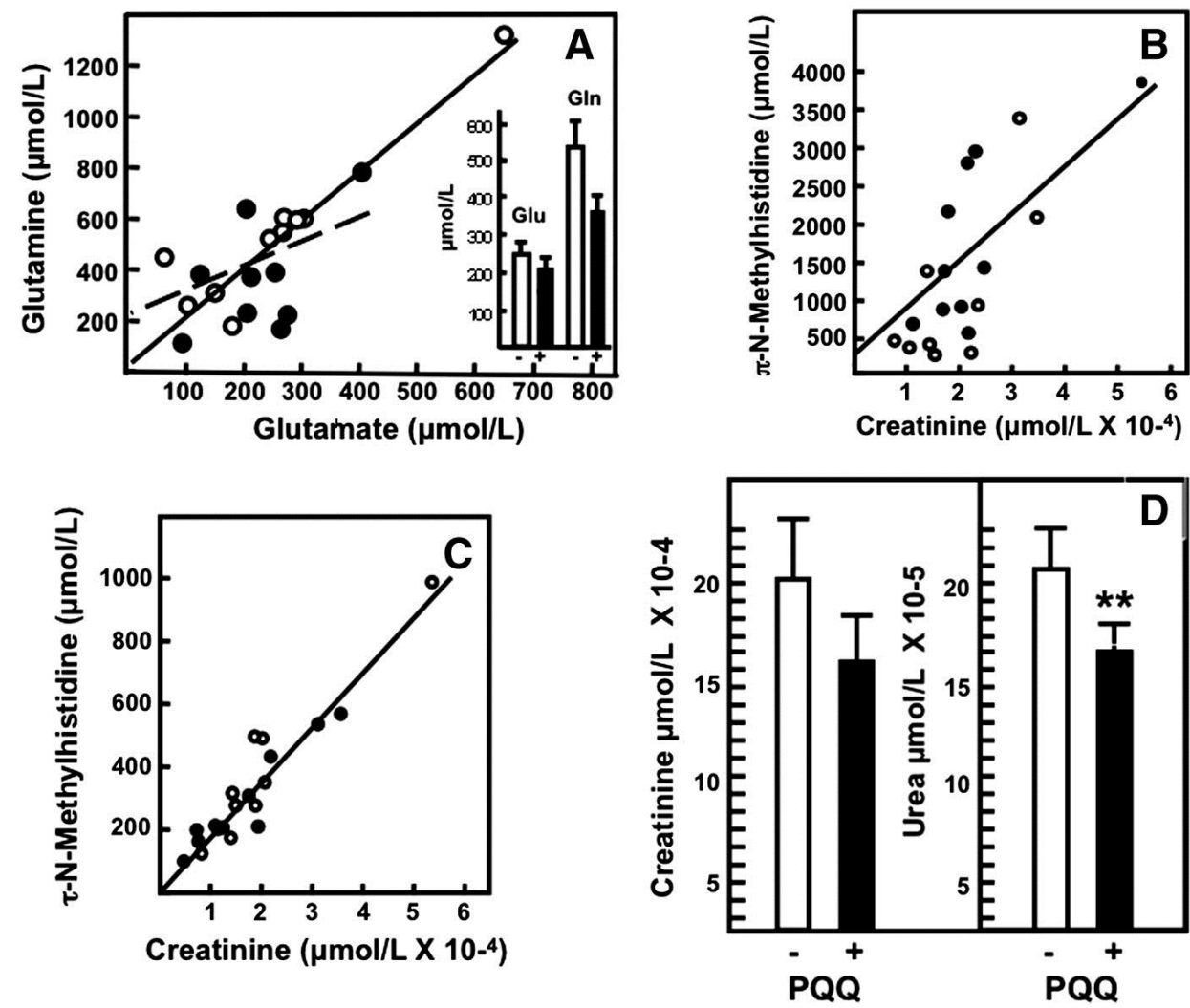
[PLoS One. 2011; 6\(7\): e21779.](https://doi.org/10.1371/journal.pone.021779)



STZ-treated animals received PQQ (mg/kg body mass/day) for 15 days), significantly decreased the serum levels of glucose and lipids.

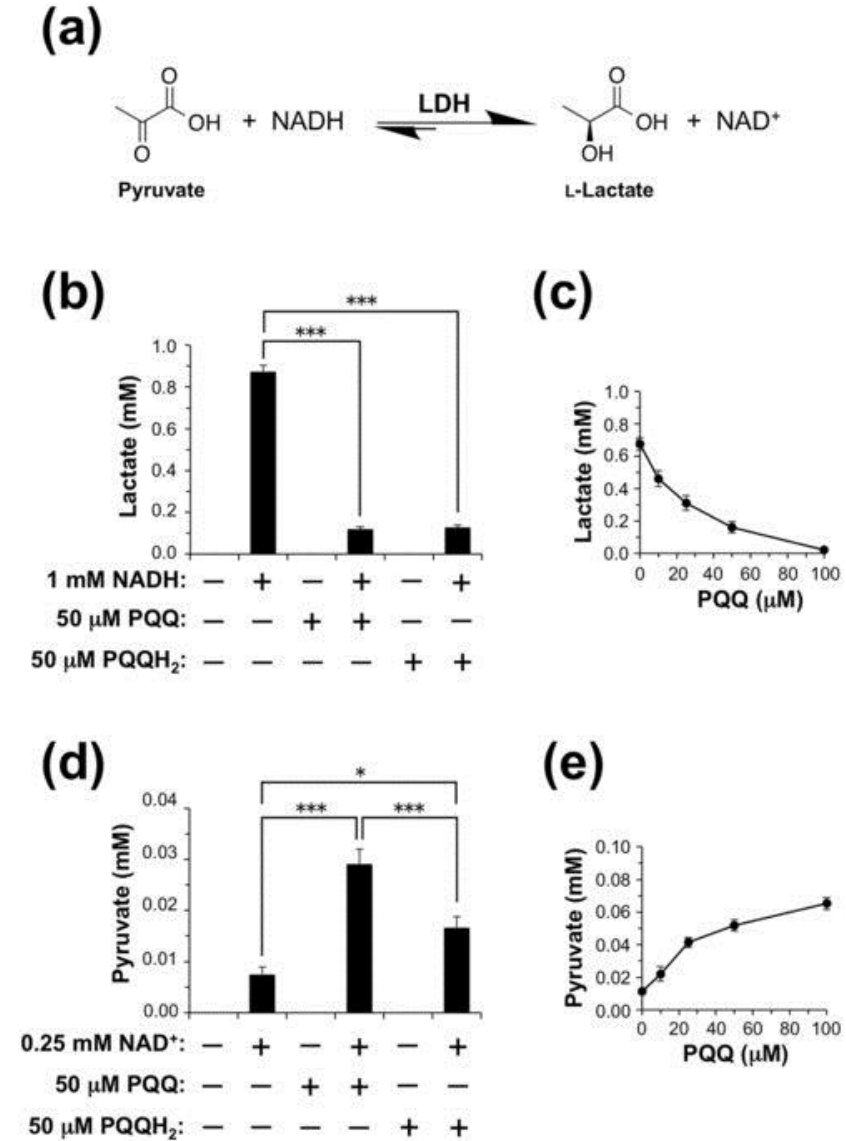
[Canadian Journal of Physiology and Pharmacology 93\(1\):1-9 · 2014](https://doi.org/10.1155/2014/123456)

PQQ and indices of muscle protein retention



[J Nutr Biochem.](#) 2013 Dec;24(12):2076-84. Dietary pyrroloquinoline quinone (PQQ) alters indicators of inflammation and mitochondrial-related metabolism in human subjects

Regulation of LDH activity by PQQ

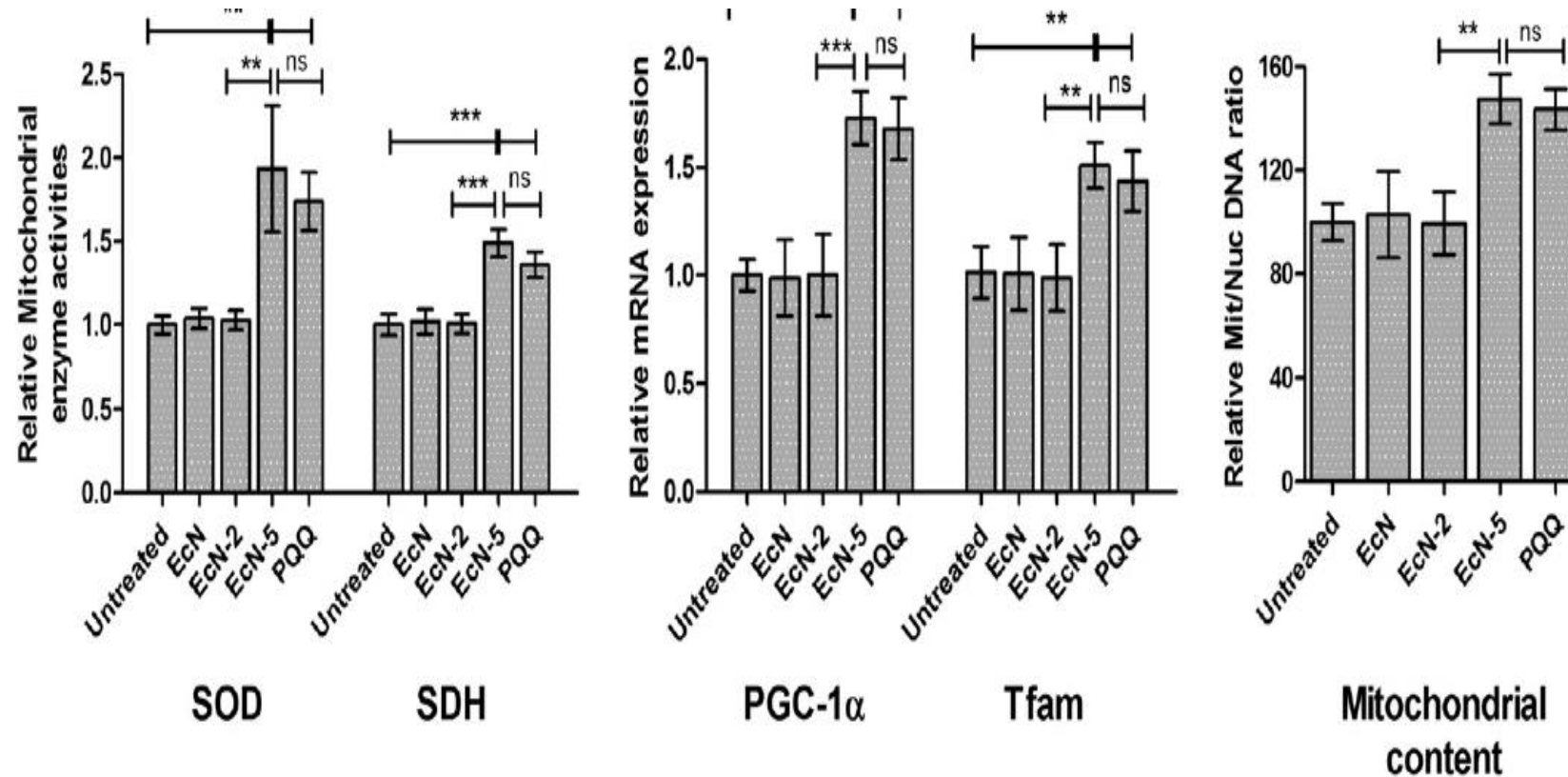


[Sci Rep.](#) 2016 May 27;6:26723. Identification of lactate dehydrogenase as a Mammalian pyrroloquinoline quinone (PQQ)-binding protein

Pyrroloquinoline quinone (PQQ) producing *Escherichia coli* Nissle 1917 (EcN) alleviates age associated oxidative stress and hyperlipidemia, and improves mitochondrial function in ageing rats

- The present work demonstrates the protective effect of PQQ producing EcN against rotenone induced mitochondrial oxidative stress and consequence of mitochondrial and cellular dysfunction in naturally ageing rat model.
- First adult rats (16-18 weeks old) were treated with rotenone (2.5 mg/kg body weight; i.p.) daily for 28 days along with PQQ (10 mg/kg diet, daily) and modified probiotic EcN strains (10(8) CFU twice weekly). Secondly, ageing rats (48-50 weeks old) were gavaged with probiotic EcN strains (10(8)CFU twice weekly) and PQQ (10 mg/kg diet, daily) for 8 months.
- EcN-5 treatment prevented rotenone induced hepatic oxidative stress and mitochondrial damage in rats as assessed by reduced lipid peroxidation (29%), elevated glutathione (GSH) content (43%), increased c
- Moreover, increased hepatic mitochondrial content (41%), peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1 α) mRNA (25%) and mitochondrial Superoxide Dismutase (Mit-SOD) activity (94%) were also observed in EcN-5 treated rats.
- Rotenone treated rats did not exhibit gain in body weight, whereas rats co-treated with EcN-5 showed significant restoration in body weight gain.
- Weekly administration of EcN-5 to naturally ageing rats for eight months resulted in significant reduction of oxidative stress in hepatic and colonic tissues (assessed by lipid peroxidation, GSH content and catalase and SOD enzyme activities) along with increase in hepatic mitochondrial enzyme activities (Mit-SOD and succinate dehydrogenase) and biogenesis, when compared to untreated rats.
- These rats also exhibited reduced expression of fatty acid synthase (50%) and increased expression of acyl coenzyme oxidase (225%) genes in liver in contrast to untreated rats resulting in lowered triglyceride (13% & 13.5%) and cholesterol (21% & 27%) levels in plasma and liver, respectively.
- Increased levels of butyrate (93%), propionate (45%) and acetate (18%) were also found in colonic content of these rats.
- PQQ administered daily (supplemented in diet) exhibited more or less similar effect as weekly gavaged EcN-5 in both the experiments, which substantiate that these effects are mediated by PQQ.
- Exp Gerontol. 2015 Jun;66:1-9.

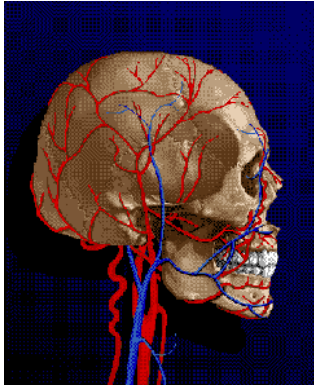
PQQ improves mitochondrial function in ageing rats



8 months feeding assay

Pyrroloquinoline quinone (PQQ) producing *Escherichia coli* Nissle 1917 (EcN) alleviates age associated oxidative stress and hyperlipidemia, and improves mitochondrial function in ageing rats

- PQQ/EcN-5 prevented hepatic oxidative stress against rotenone.
- PQQ/EcN-5 reduced oxidative stress and restored lipid profile in naturally ageing rats.
- PQQ/EcN-5 increased mitochondrial biogenesis and metabolism in naturally ageing rats.
- PQQ/EcN-5 can serve as nutritive supplement to delay ageing.
- PQQ administered daily exhibited similar effect as weekly gavaged EcN-5 in both the experiments, which substantiate that these effects are mediated by PQQ



**Ischemic
Stroke**



**Hypoglycemia
& Hypoxia**



Loss of cell homeostasis,
cell death by two paths:
necrosis and apoptosis

(Martin, 1998)

Reperfusion

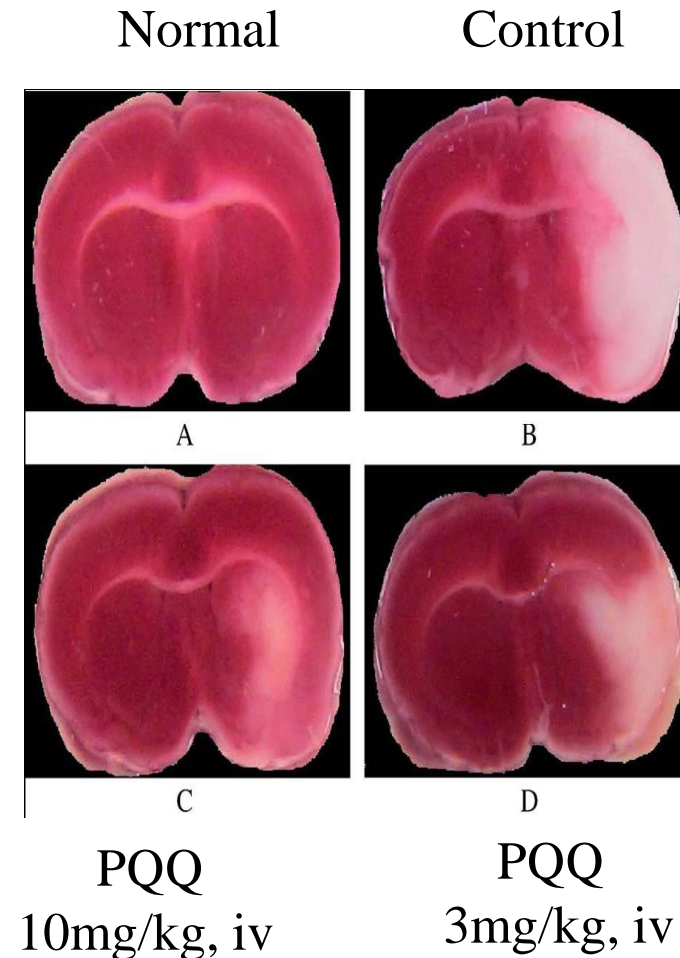
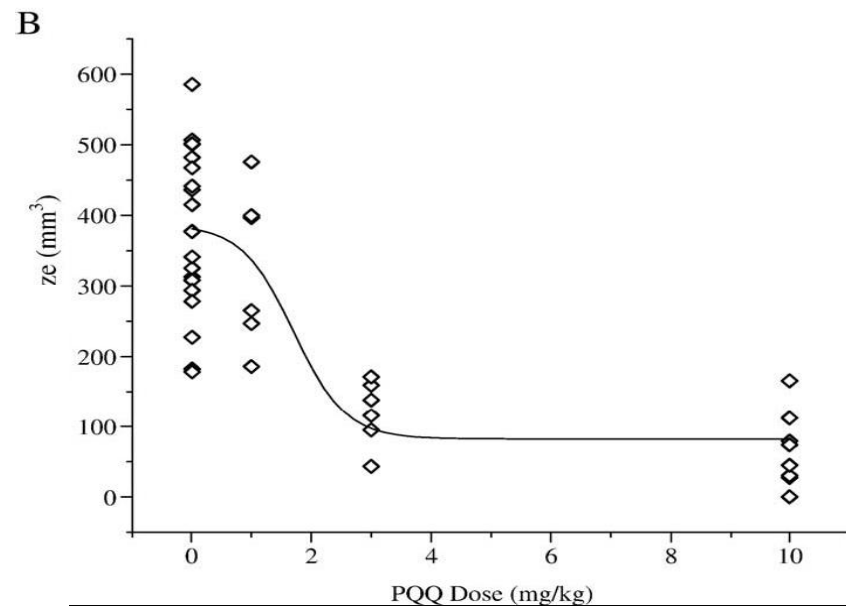
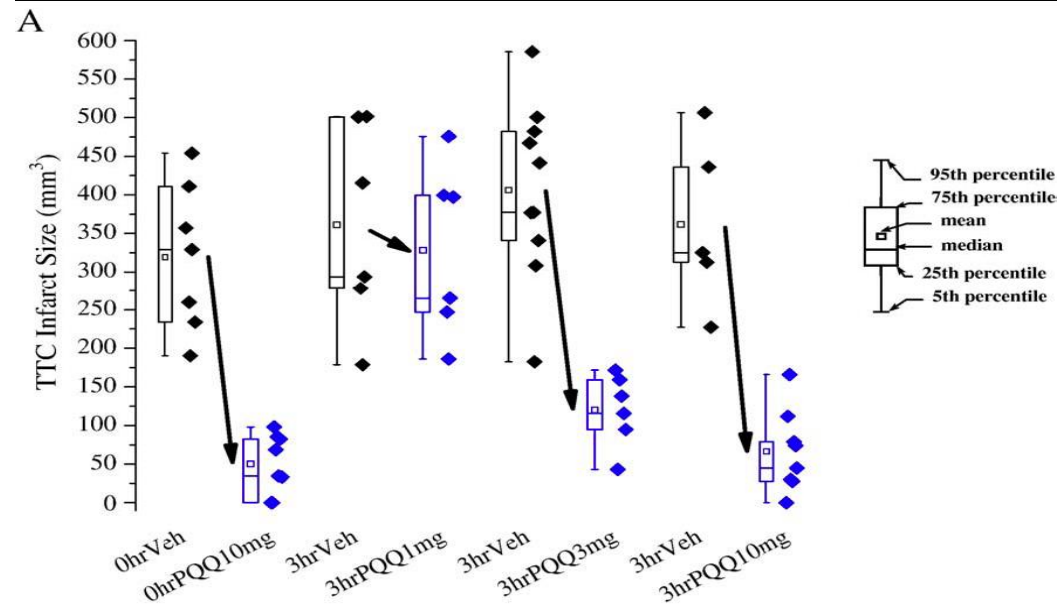


**Reactive oxygen
species
[O₂^{-•}, H₂O₂]**



**Oxidative
damage, cell
death** (Chan, 2001)

PQQ Helps to Rescue Ischemia



Zhang Y et al. [Brain Res.](#) 2006 ;1094(1):200-6.

PQQ inhibits the amyloid fibril formation and cytotoxicity of the C-terminal truncated alpha-synuclein variants

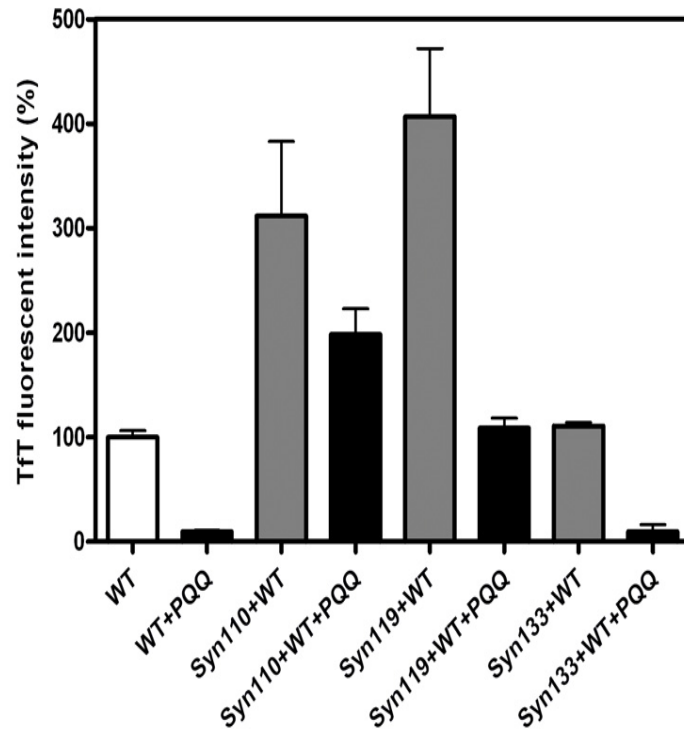


Figure 8 Inhibition by PQQ of the amyloid fibril formation of C-terminal-truncated α -synuclein mixed with full-length α -synuclein. 70 μ M full-length α -Syn or a mixture of 35 μ M full-length α -Syn and 35 μ M truncated variants was incubated in PBS buffer, pH 7.4 with stirring at 37°C in the absence (white bar or gray bar) and in the presence of 280 μ M PQQ (black bar). Fibril formation was measured by Tft fluorescence.

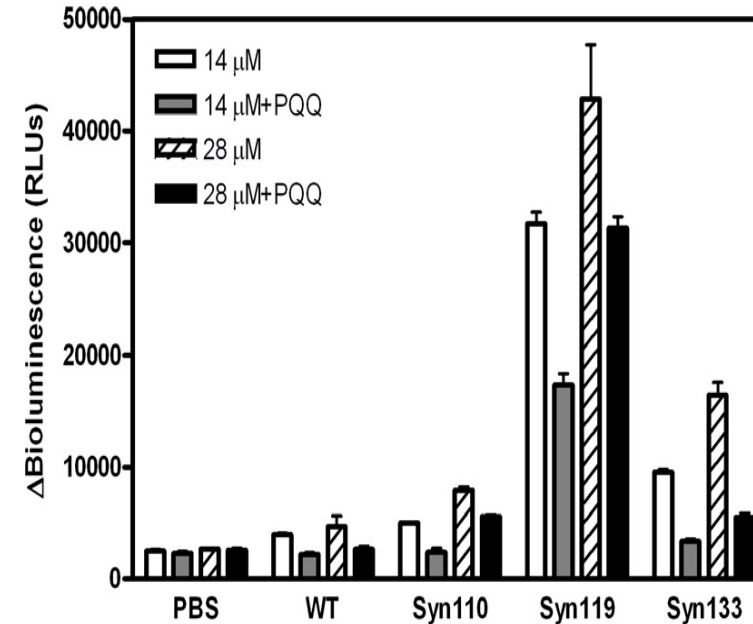
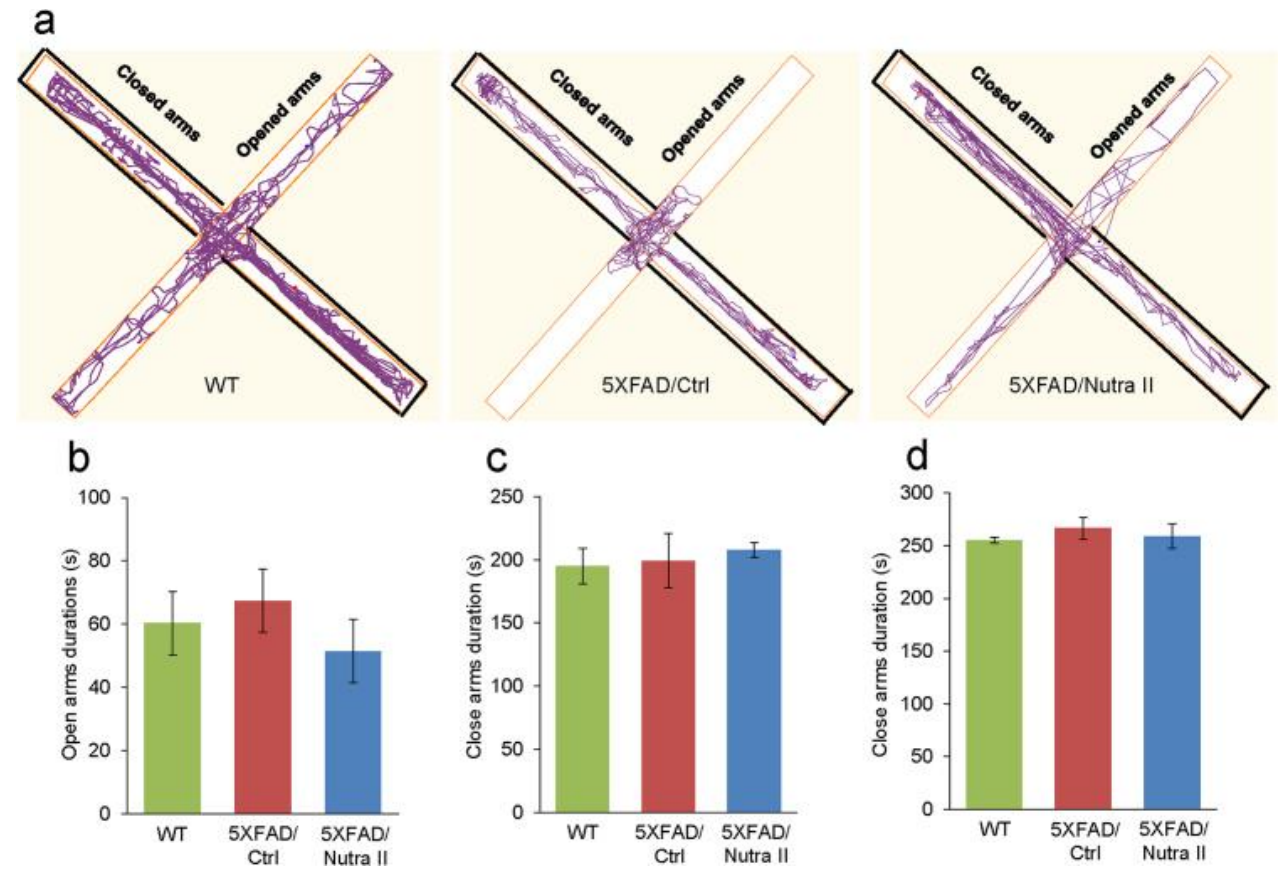


Figure 9 C-terminal-truncated α -synuclein-mediated cytotoxicity can be mitigated by PQQ. Full-length α -Syn and truncated α -Syn (final concentration 14 or 28 μ M) were added to PC12 cells with shaking at 37°C in the absence or presence of 200 μ M PQQ. After 96 h of incubation, the release of adenylate kinase from the damaged cells was measured by the luminescence of luciferase. $n = 3$ and error bar = standard deviation.

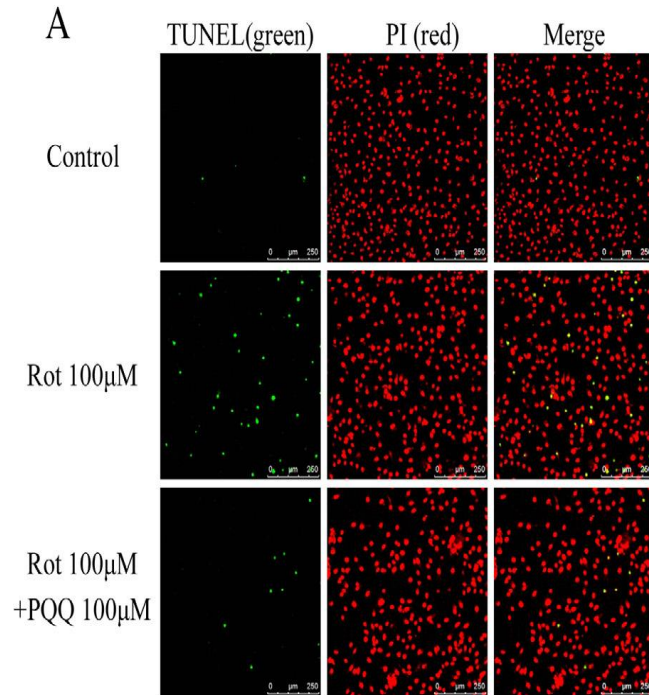
[Heliyon](#). 2017 Apr 4;3(4):e00279.

Beneficial effects of a pyrroloquinolinequinone-containing dietary formulation on motor deficiency, cognitive decline and mitochondrial dysfunction in a mouse model of Alzheimer's disease.

Alzheimer's disease (AD), a progressive neurodegenerative disorder, is linked to oxidative stress, altered amyloid precursor protein (APP) proteolysis, tau hyperphosphorylation and the accumulation of amyloid- β (A β) plaques and neurofibrillary tangles (NFT). A growing body of evidence suggests **that mitochondrial dysfunction can be a key promoter** of all of these pathologies and predicts that restoration of mitochondrial function might be a potential therapeutic strategy for AD. Therefore, in the present study, we tested the beneficial effect of a nutraceutical formulation Nutrastem II (Nutra II), containing NT020 (a mitochondrial restorative and antioxidant proprietary formulation) and pyrroloquinolinequinone (PQQ, a stimulator of mitochondria biogenesis) in **5XFAD transgenic mice**. Animals were fed Nutra II for **12 weeks**, starting **at 3 months of age**, after which behavioral and neuropathological endpoints were determined. The data from behavioral test batteries clearly revealed that dietary **supplementation of Nutra II effectively ameliorated the motor deficiency and cognitive impairment of 5XFAD mice**. In addition, Nutra II also **protected mitochondrial function** in 5XFAD mice brain, as evidenced by declined ROS levels and membrane hyperpolarization, together with elevated ATP levels and respiratory states. Interestingly, while Nutra II treatment only slightly reduced soluble A β_{42} levels, this formulation significantly impacted tau metabolism, as shown by reduced total and phosphorylated tau levels of 5XFAD mouse brain. Taken together, these preclinical findings confirm that **mitochondrial function may be a key treatment target for AD** and that Nutra II should be further investigated as a potential candidate for AD therapy.



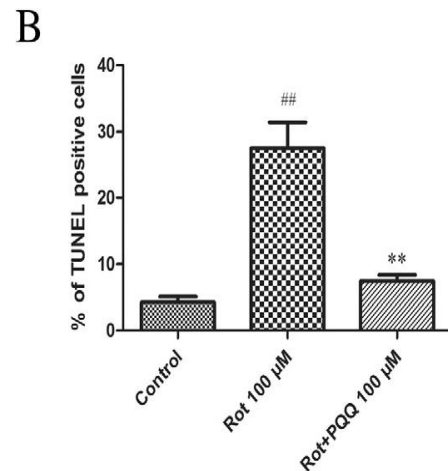
PQQ Against Rotenone-Induced SH-SY5Y Cell injury



In vitro model of Parkinson's disease (PD) by exposing cultured **SH-SY5Y dopaminergic cells** to rotenone, a complex I inhibitor.

The neuroprotective effects of PQQ were observed by pretreatment of SH-SY5Y cells with PQQ before rotenone injury.

PQQ pretreatment prevented SH-SY5Y cells from rotenone-induced apoptosis in a concentration dependent manner.

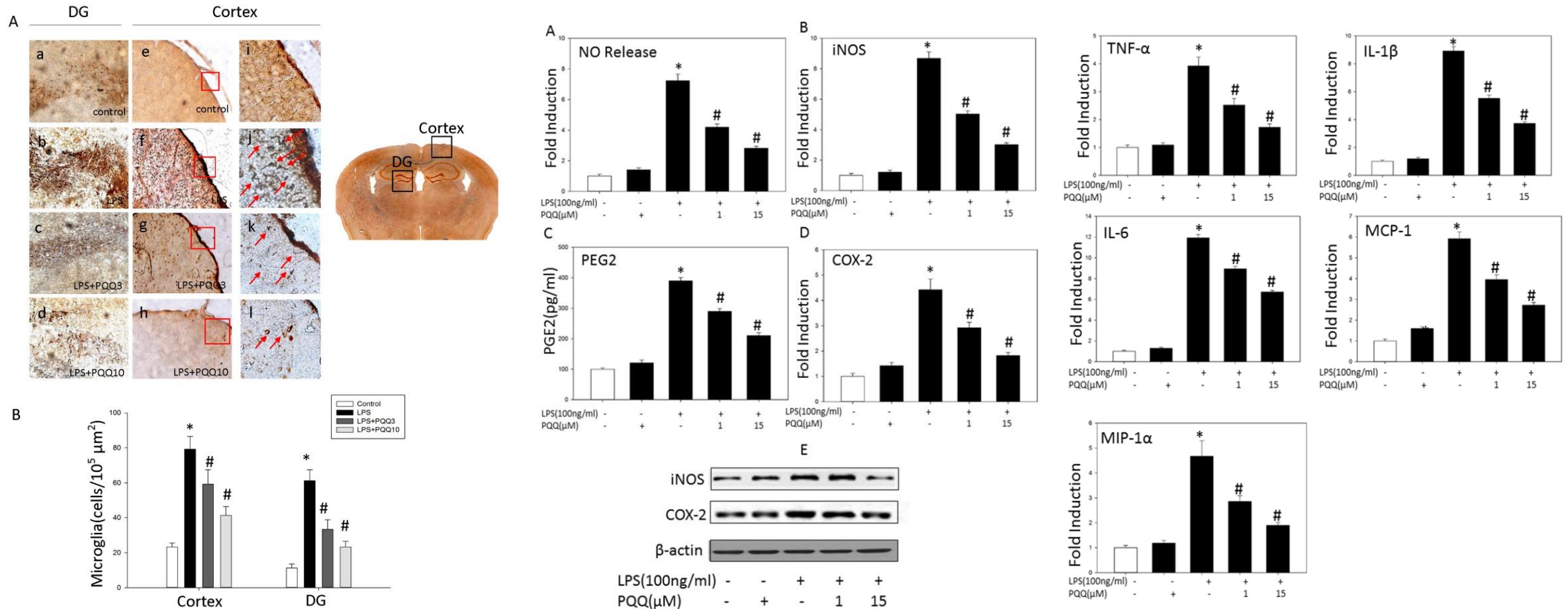


TUNEL assay was applied to detect the percentage of apoptotic (TUNEL-positive) cells in total cell population

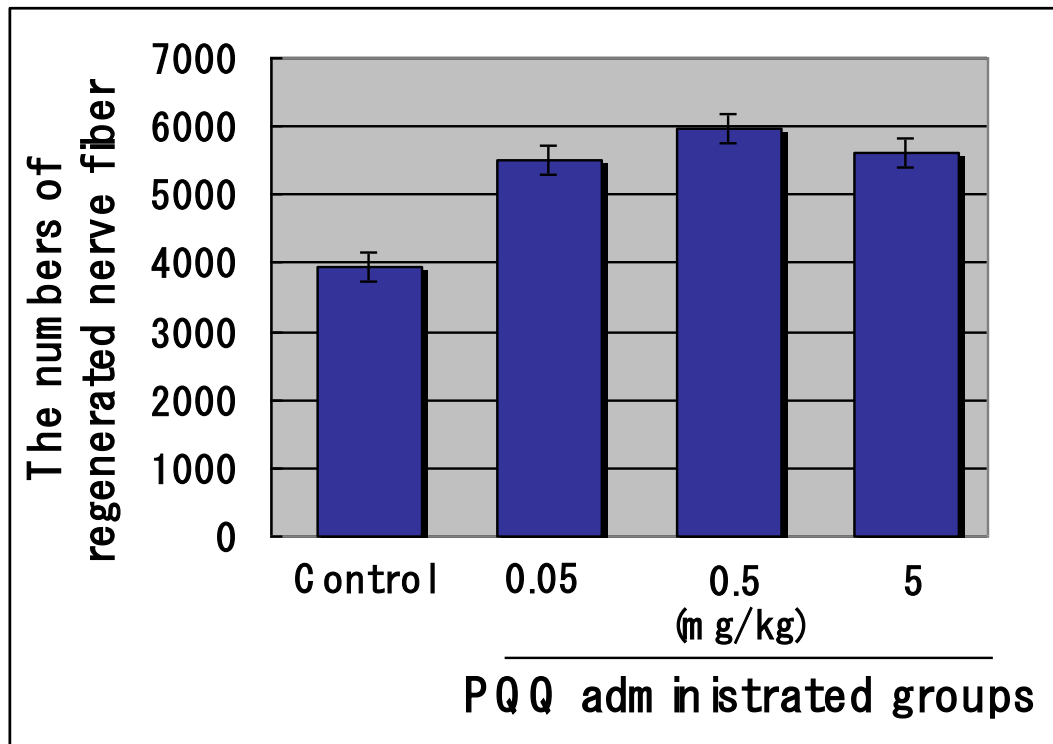
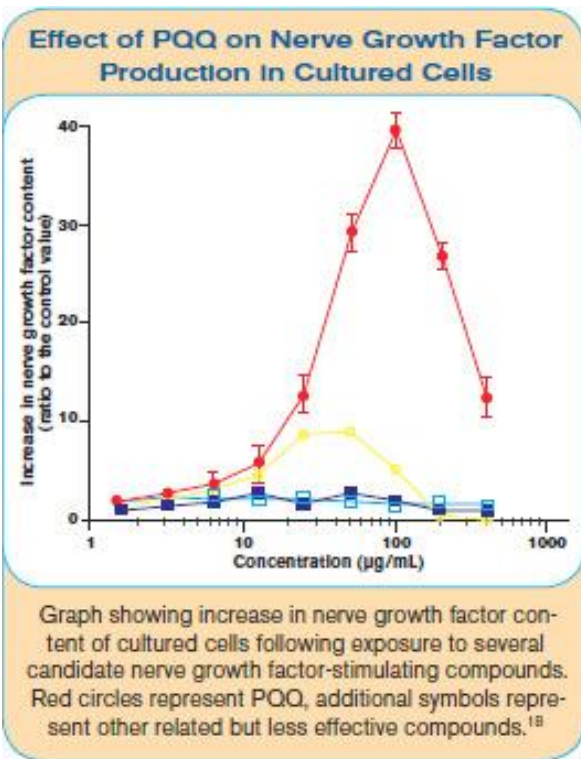
Q. Zhang et al. / Neuroscience 270 (2014)



Pyrroloquinoline quinone (PQQ) inhibits lipopolysaccharide induced inflammation in part via downregulated NF- κ B and p38/JNK activation in microglial and attenuates microglia activation in lipopolysaccharide treatment mice. PLoS One. 2014 Oct 14;9(10):e109502



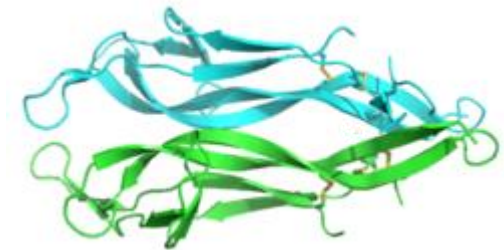
Improvement of Peripheral Neuropathy with PQQ



Oral administration for 2 wks in rat

Koyama T. et al. (2006)

Nerve Growth Factor (NGF) is a protein that is important for the growth, maintenance, and survival of neurons.



Evidences of PQQ Protects the Brain

- **PQQ Promotes *New* Mitochondrial Formation**
- **PQQ Promotes Nerve Cell Growth**
- **PQQ Protects against Oxidative Damage**
 - PQQ led to significantly improved neurobehavioral scores after the stroke
- **PQQ Reduces Harmful Neuroinflammation**
- **PQQ Protects against Excitotoxicity**
 - not only can PQQ help protect against the damaging effects of excitotoxicity, it can also help prevent it from occurring to begin with
- **PQQ Prevents Glucose-Induced Brain Damage**
 - PQQ significantly *reversed* brain cell damage in diabetic mice
- **PQQ Inhibits Malformed Brain Proteins**
 - Excitement is growing in the scientific community about PQQ's ability to inhibit the formation of toxic protein fibrils in both Alzheimer's and Parkinson's diseases.

Cardiovascular System

PQQ decrease the infarct size after cardiac infarct

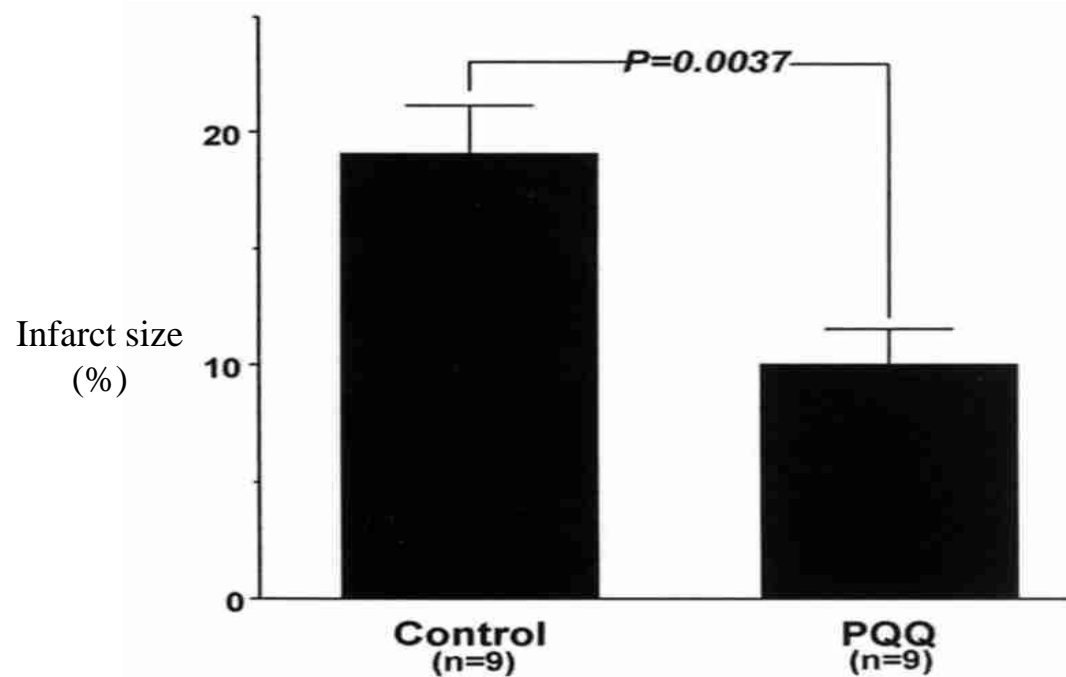


Table 2 PQQ Cardiac Protective Effects				
Parameters	Administration <u>before</u> heart attack		Administration <u>after</u> heart attack	
	(-) PQQ	(+) PQQ	(-) PQQ	(+) PQQ
% survived	88.9%	100.0%	62.5%	100.0%

Data: Baurely K, et al. 2011

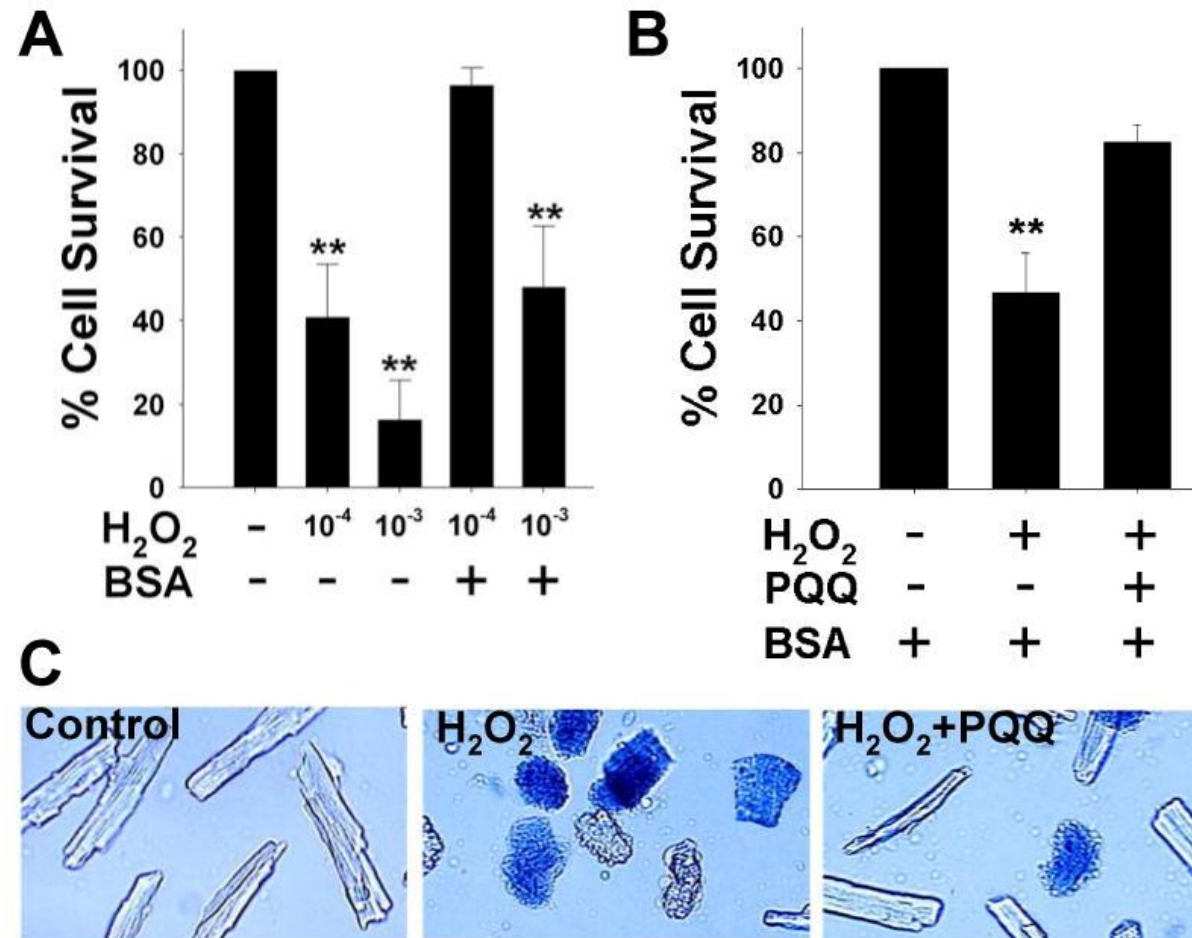


Zhu B et al. [Cardiovasc Drugs Ther.](#) 2004 Nov;18(6):421-31.

We conclude that PQQ, which appears to act as a free radical scavenger in ischemic myocardium, is a highly effective cardioprotective agent

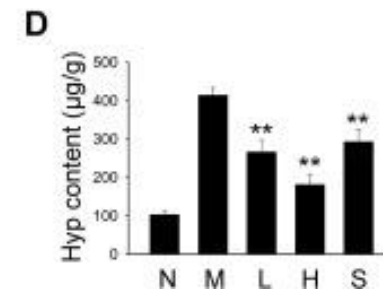
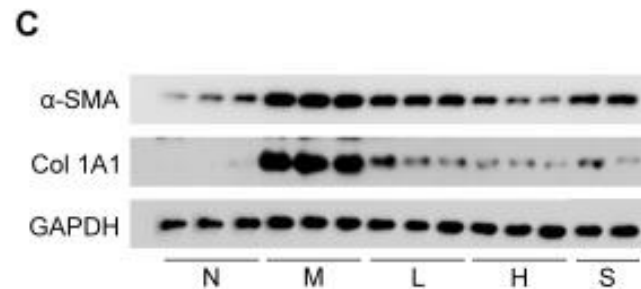
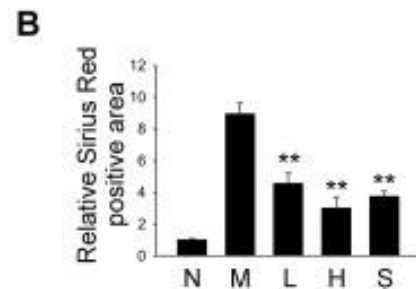
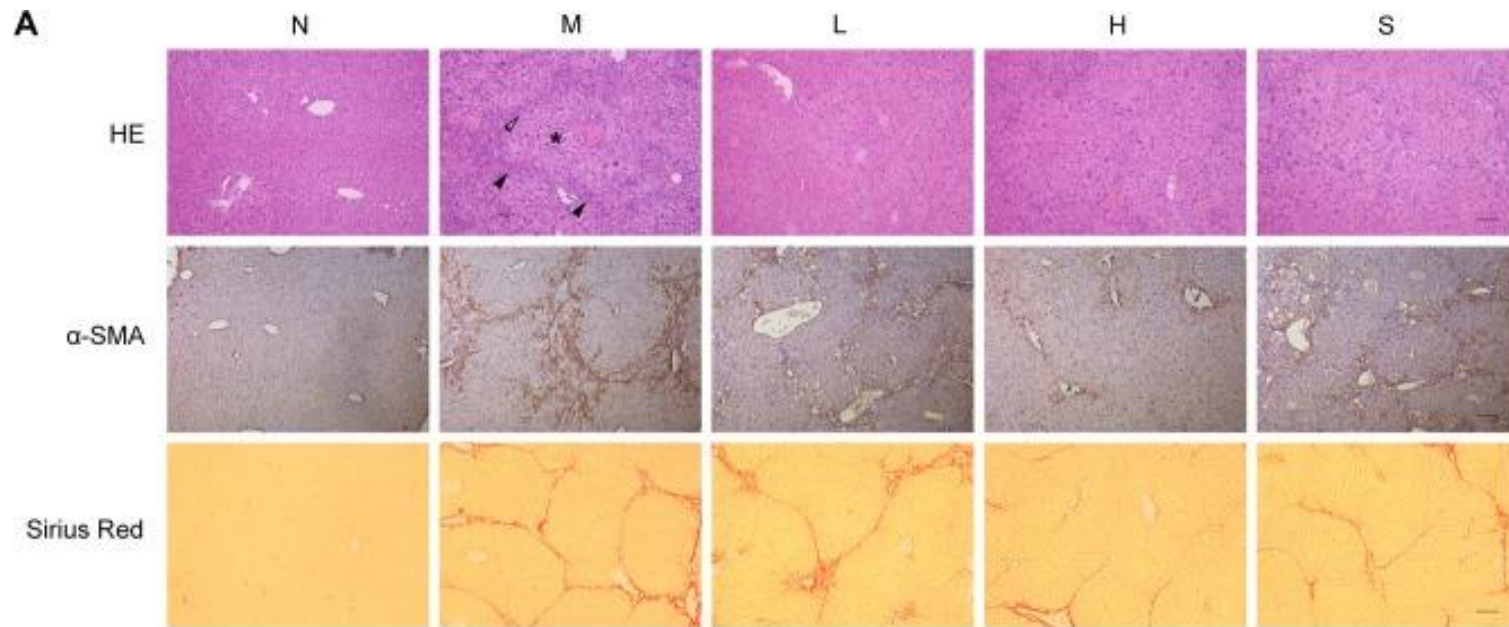
[Biochem Biophys Res Commun.](#) 2007 Nov 16;363(2):257-62.

Pyrroloquinoline quinone preserves mitochondrial function and prevents oxidative injury in adult rat cardiac myocytes.

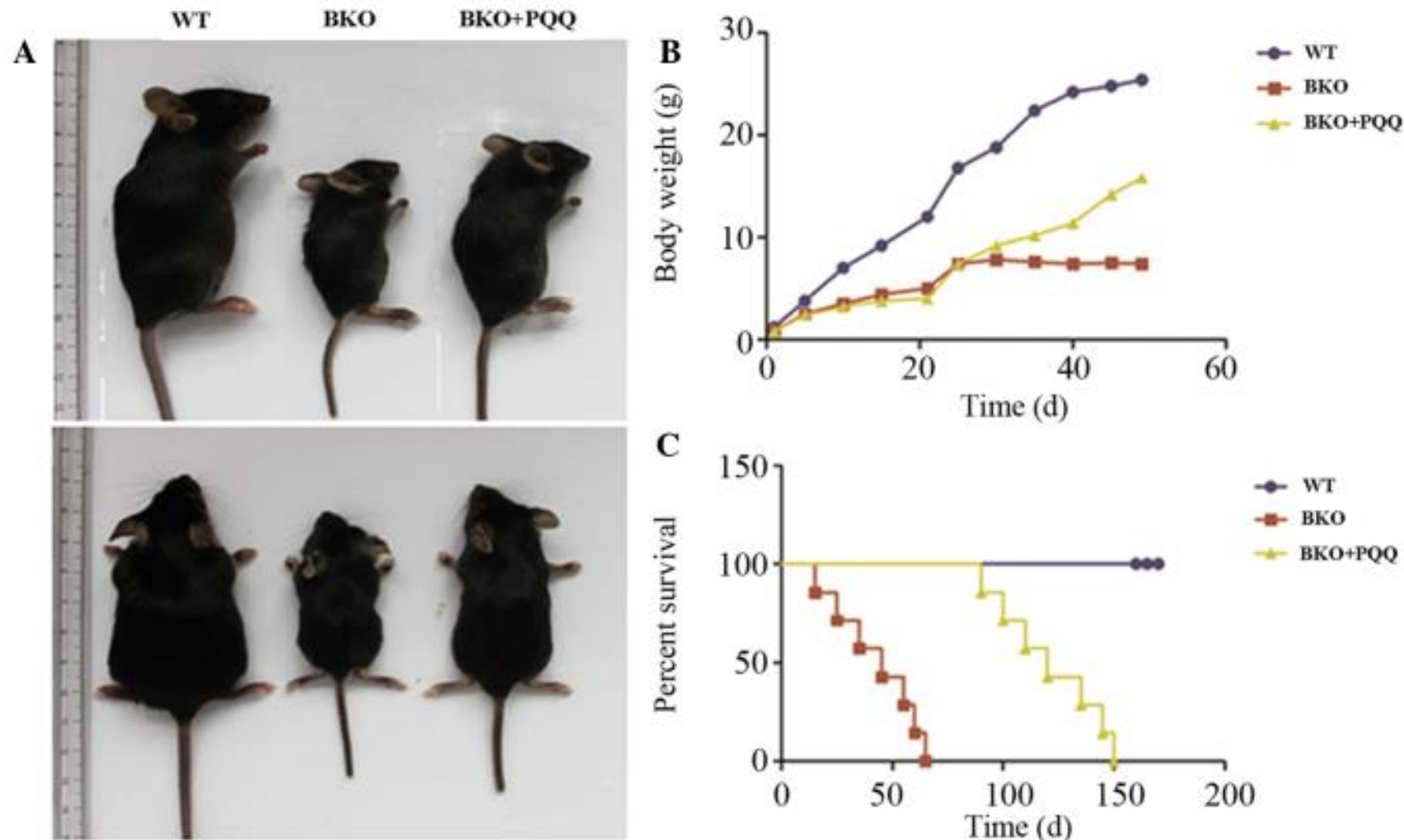


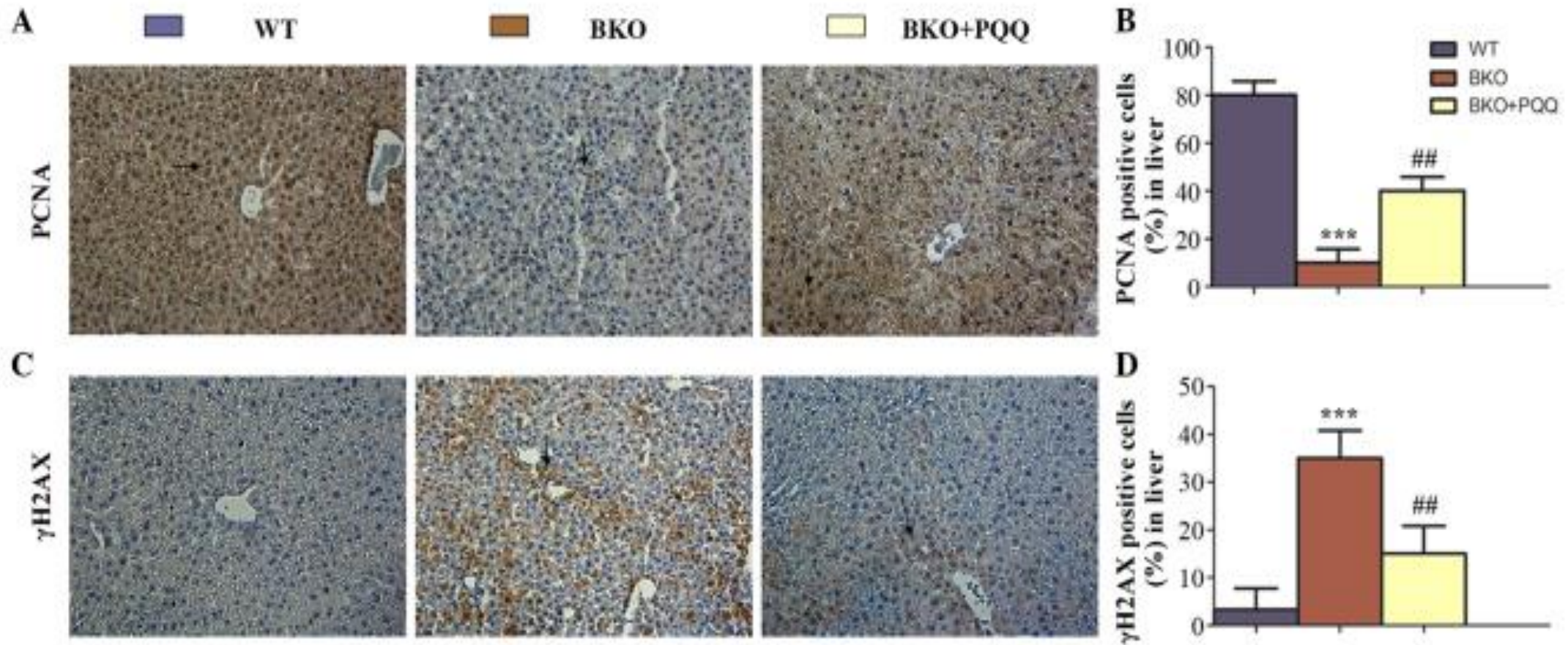
Liver Protection

PQQ Rescue TAA-induced Liver Fibrosis



PQQ is able to rescue premature senescence in the liver, induced by the deletion of B cell-specific Moloney MLV insertion site-1 (Bmi-1), by inhibiting oxidative stress.
Exp Ther Med. 2015 Aug; 10(2): 451–458.





Effects of PQQ on cell proliferation and DNA damage in the liver of BKO mice. (A) Representative liver tissues of the WT, BKO and BKO + PQQ mice stained immunohistochemically for PCNA. (B) Percentage of liver cells stained positive for PCNA in the WT, BKO and BKO + PQQ mice. (C) Representative liver tissues of the WT, BKO and BKO + PQQ mice stained immunohistochemically for γ H2AX. (D) Percentage of liver cells stained positive for γ H2AX in the WT, BKO and BKO + PQQ groups. *Exp Ther Med.* 2015 Aug; 10(2): 451–458.

[Alcohol Clin Exp Res.](#) 2014 Jul;38(7):2127-37.

Pyrroloquinoline quinone-secreting probiotic Escherichia coli Nissle 1917 ameliorates ethanol-induced oxidative damage and hyperlipidemia in rats.

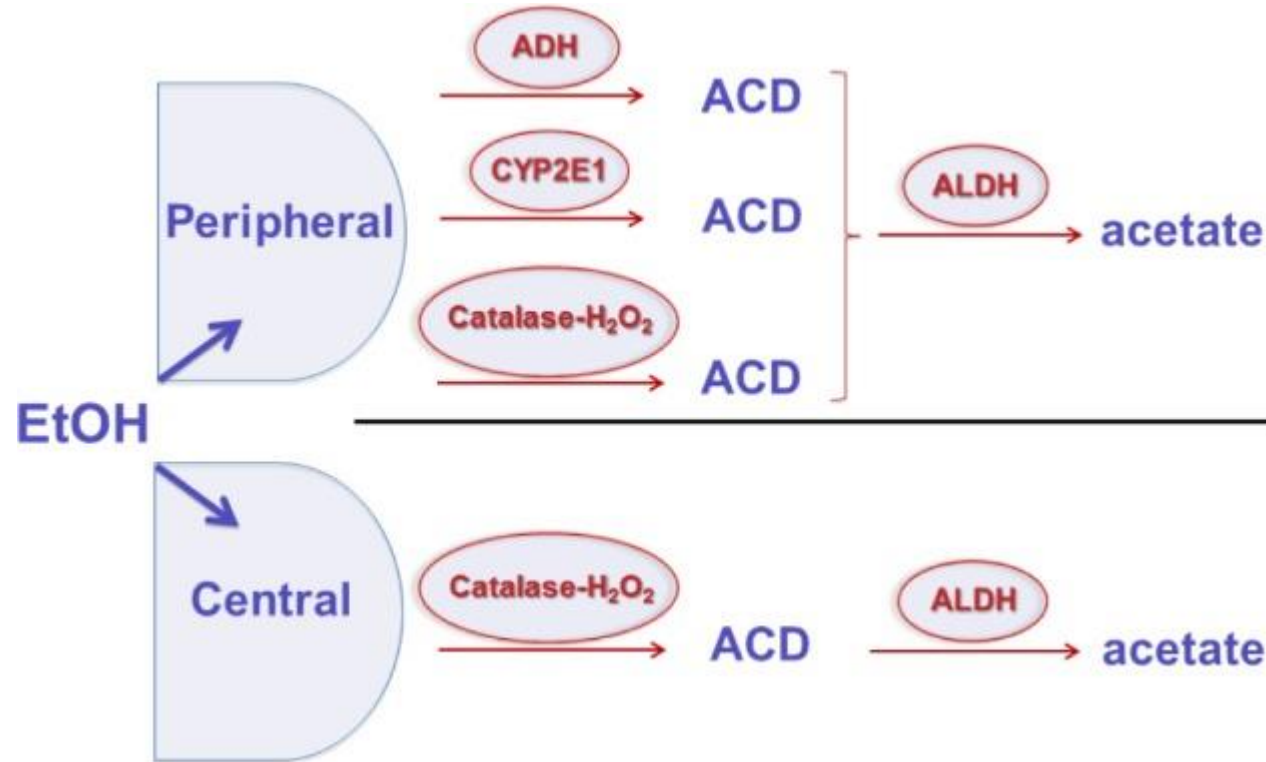
- **METHODS:** Male Charles Foster rats were gavaged with EtOH (5 g/kg body weight [acute study] and 3 g/kg body weight per day for 10 weeks [chronic study]).
- **RESULTS:** Pretreatment of PQQ, vitamin C, and PQQ-secreting EcN prevented acute EtOH-induced oxidative damage in rats reflected by reduced lipid peroxidation in blood and liver and increased hepatic reduced glutathione.
 - In the acute study, PQQ given externally was found to be most effective against acute EtOH toxicity.
 - In the chronic study, rats treated with PQQ-secreting EcN showed remarkable reduction in oxidative tissue damage (liver, colon, blood, and kidney) with significant increase in antioxidant enzyme activities as compared to only EtOH-treated rats.
 - Antioxidant and hyperlipidemic effects of PQQ-secreting EcN are correlated with increased colonic short chain fatty acids (SCFAs; i.e., acetate, propionate, and butyrate) levels, and PQQ concentration in fecal samples (2-fold) and liver (4-fold).
 - **PQQ and vitamin C given once a week, did not exhibit any ameliorative effect** against chronic EtOH toxicity.
- **CONCLUSIONS:**
 - Accumulated PQQ in tissues prevents hepatic and systemic oxidative damage.
 - **PQQ along with SCFAs reduced hyperlipidemia**, which can be correlated with changes in mRNA expression of hepatic lipid metabolizing genes.
 - Our study suggests that endogenous generation of PQQ by EcN could be an effective strategy in preventing alcoholic liver disease.

[Pharmacology.](#) 1988;37(4):264-7.

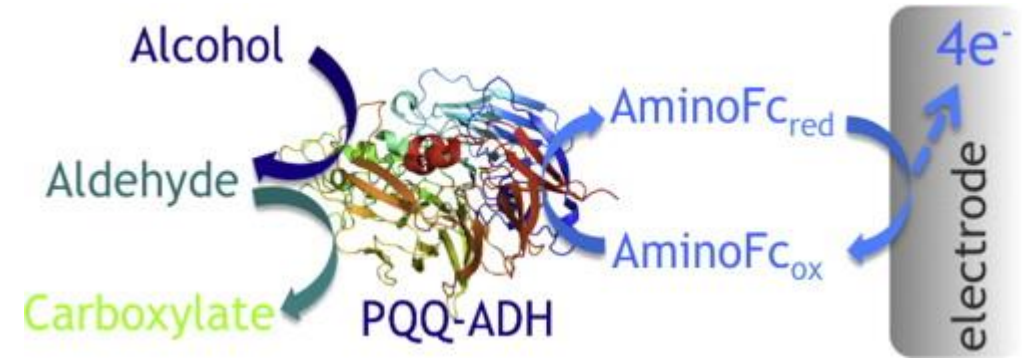
Quinone derivatives lower blood and liver acetaldehyde but not ethanol concentrations following ethanol loading to rats.

- A rise in blood and liver acetaldehyde concentrations following an intragastric administration of ethanol to rats was significantly inhibited when Coenzyme Q10, PQQ and Idebenone were injected intraperitoneally, prior to ethanol load, at a dose of 10, 11.5 and 30 mg/kg of body weight, respectively.
- When acetaldehyde was incubated in vitro with 1,4-benzoquinone (3.7-13.0 mM) or PQQ (1.4-4.9 mM) at 0 and 40 degrees C, the acetaldehyde concentrations slowly decreased with incubation time at 40 degrees C.
- The results suggest that low acetaldehyde concentrations following ethanol load are due to an **accelerated oxidation** of **acetaldehyde by PQQ** in the liver and the circulating blood.

PQQ and Ethanol Metabolism



ACD, acetaldehyde; EtOH, ethanol; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P₄₅₀, isoform 2E1.

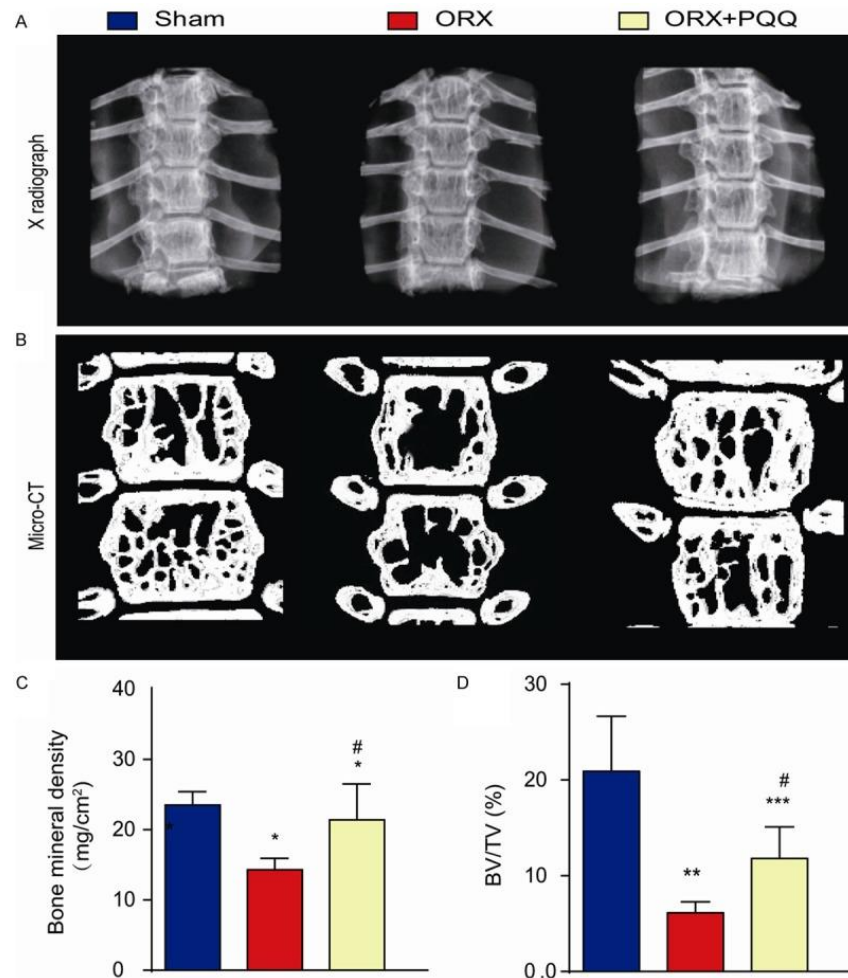


- Quinoprotein alcohol dehydrogenases use the pyrroloquinoline quinone (PQQ) cofactor to catalyze the oxidation of alcohols. [J Biol Chem.](#) 2006 Jan 20;281(3):1470-6.
- **PQQ** was found to be most effective against acute EtOH toxicity. [Alcohol Clin Exp Res.](#) 2014 Jul;38(7):2127-37
- In the chronic study, accumulated PQQ in tissues prevents hepatic and systemic oxidative damage. [Alcohol Clin Exp Res.](#) 2014 Jul;38(7):2127-37.
- Fresh **orange juice** significantly increased the content of acetaldehyde in blood as well as the activities of AST and ALT, and remarkably inhibited the activity of ALDH. [Int J Mol Sci.](#) 2016 Mar; 17(3): 354.
- Soda water, **green tea**, and honey **chrysanthemum** tea are recommended to consume accompanied with alcohol drinking due to their capacities of accelerating ethanol metabolism and preventing liver injuries caused by alcohol . [Int J Mol Sci.](#) 2016 Mar; 17(3): 354.

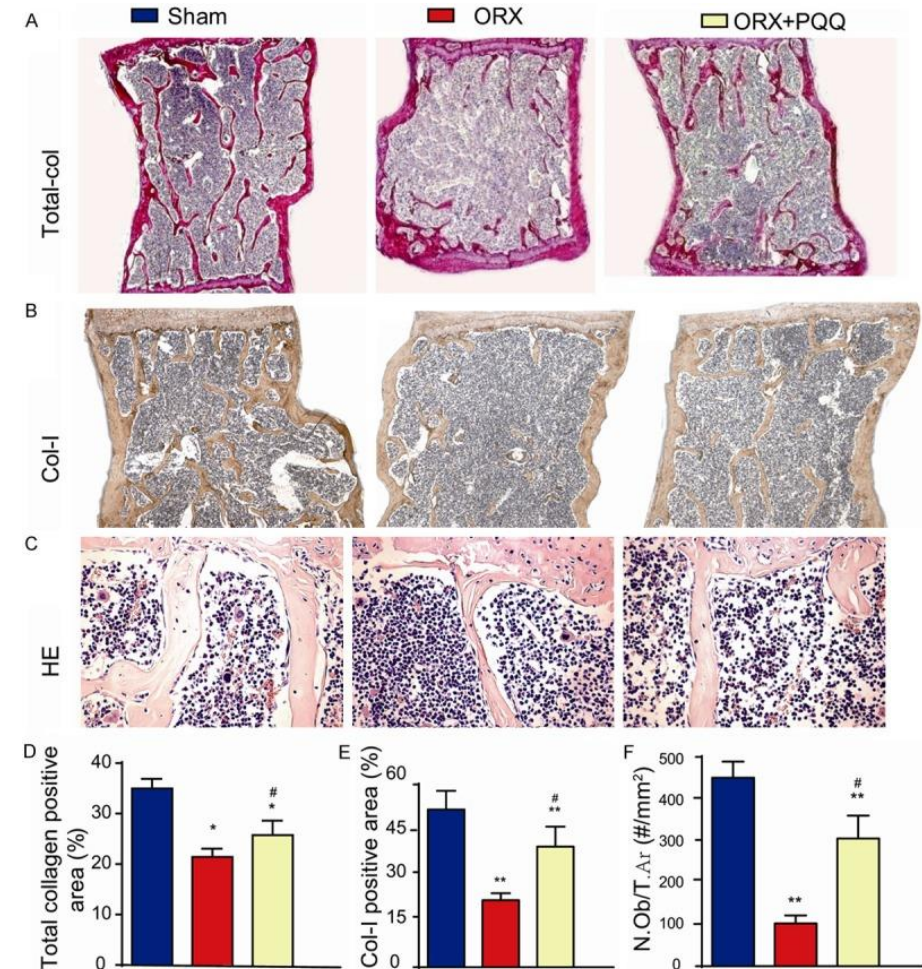
PQQ for joint health

- [PLoS One](#). 2013 Apr 17;8(4): **Pyrroloquinoline Quinine Inhibits RANKL-mediated Expression of NFATc1 in Part via Suppression of c-Fos in Mouse Bone Marrow Cells and **Inhibits** Wear Particle-induced **Osteolysis** in Mice.**
- [Inflammation](#). 2015 Aug;38(4):1546-55. **Pyrroloquinoline Quinone Slows Down the Progression of Osteoarthritis by Inhibiting Nitric Oxide Production and Metalloproteinase Synthesis.**
- [Inflammation](#). 2016 Feb;39(1):248-56. **Pyrroloquinoline Quinone Decelerates Rheumatoid Arthritis Progression by Inhibiting Inflammatory Responses and Joint Destruction via Modulating NF-κB and MAPK Pathways.**
- [Eur J Pharmacol](#). 2012 Dec 15;697(1-3):53-8. **Effect of Pyrroloquinoline Quinone on Neuropathic Pain following Chronic Constriction Injury of the Sciatic Nerve in Rats.**
- [Am J Transl Res](#). 2017; 9(3): 1230–1242. **Pyrroloquinoline quinone prevents testosterone deficiency-induced osteoporosis by stimulating osteoblastic bone formation and inhibiting osteoclastic bone resorption.**

Pyrroloquinoline quinone prevents testosterone deficiency-induced osteoporosis by stimulating osteoblastic bone formation and inhibiting osteoclastic bone resorption. [Am J Transl Res. 2017; 9\(3\): 1230–1242.](#)



Effect of PQQ on ORX-induced osteoporosis. 2-month-old C57BL/6J wild-type mice were received ORX surgery at both sides or sham surgery. PQQ supplementary diet was given to ORX mice after surgery. T9-T12 vertebrae were harvested 48 weeks after PQQ treatment. A. Representative X-ray images. B. Representative micro-CT scans. C. Bone density by densitometry analysis on X-ray images. D. Bone volume (BV/TV) from micro-CT analysis. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, vs sham group; #, $P < 0.05$, vs ORX mice.



Effect of PQQ on osteoblastic bone formation of ORX mice. (A) T12 vertebrae sections from sham-operated mice, ORX mice and PQQ treated ORX mice were stained histochemically for total collagen (×100); (B) Immunohistochemically for Col-I (×100) and (C) with H&E (×200). (D) The relative total collagen positive area to tissue area was counted from (A). (E) The relative Col-I positive area to tissue area was counted from (B). (F) The osteoblast number were counted in H&E stained vertebrae sections. *, $P < 0.05$; **, $P < 0.01$, vs sham-operated mice; #, $P < 0.05$, vs ORX mice.

PQQ Supply Status

PQQ Produced by fermentation is complex and costly.

- Fermentation technology is difficult to scale up and has low reproducibility.
- Since its introduction in 2008, the limitation of supply prevents its market growth.

Restricted supply leads to lack of marketing & public education

Only selected few have access to supply -

- co-branding & multi-year price agreements

Lack of desire to sponsor additional researches by the manufacturer.

[Appl Microbiol Biotechnol](#). 2016 Jul 28.

Novel and efficient screening of PQQ high-yielding strains and subsequent cultivation optimization.

- Using high-throughput method, PQQ high-yielding strains were rapidly screened out from thousands of methylotrophic colonies at a time.
- The comprehensive phylogenetic analysis revealed that the highest PQQ-producing strain zju323 (CCTCC M 2016079) could be assigned to a novel species in the genus *Methylobacillus* of the Betaproteobacteria.
- After systematic optimization of different medium components and cultivation conditions, about **33.4 mg/L** of PQQ was obtained after **48 h** of cultivation with *Methylobacillus* sp. zju323 at the shake flask scale.
- Further cultivations of *Methylobacillus* sp. zju323 were carried out to investigate the biosynthesis of PQQ in 10-L bench-top fermenters. In the batch operation, the PQQ accumulation reached **78 mg/L** in the broth after **53 h** of cultivation.
- By adopting **methanol feeding strategy**, the highest PQQ concentration was improved up to **162.2 mg/L** after **75 h** of cultivation (9.7kg @ 60 Tons of Methanol Incubation). This work developed a high-throughput strategy of screening PQQ-producing strains from soil samples and also demonstrated one potential bioprocess for large-scale PQQ production with the isolated PQQ strain.

ZCHT Taking the lid off

Chemical Synthesis is more cost-effective and consistent.

~60% less in cost

Ease of process expansion & controls.

Improved physical attributes – less hygroscopic, higher purity

Multiple form choice – Disodium Salt & Acid form

Continuous partnerships to explore additional benefits of PureQQ.



Shanghai Medical College
(Fudan University)

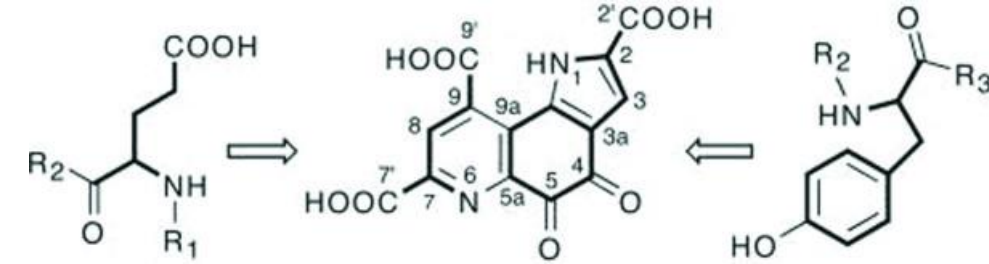
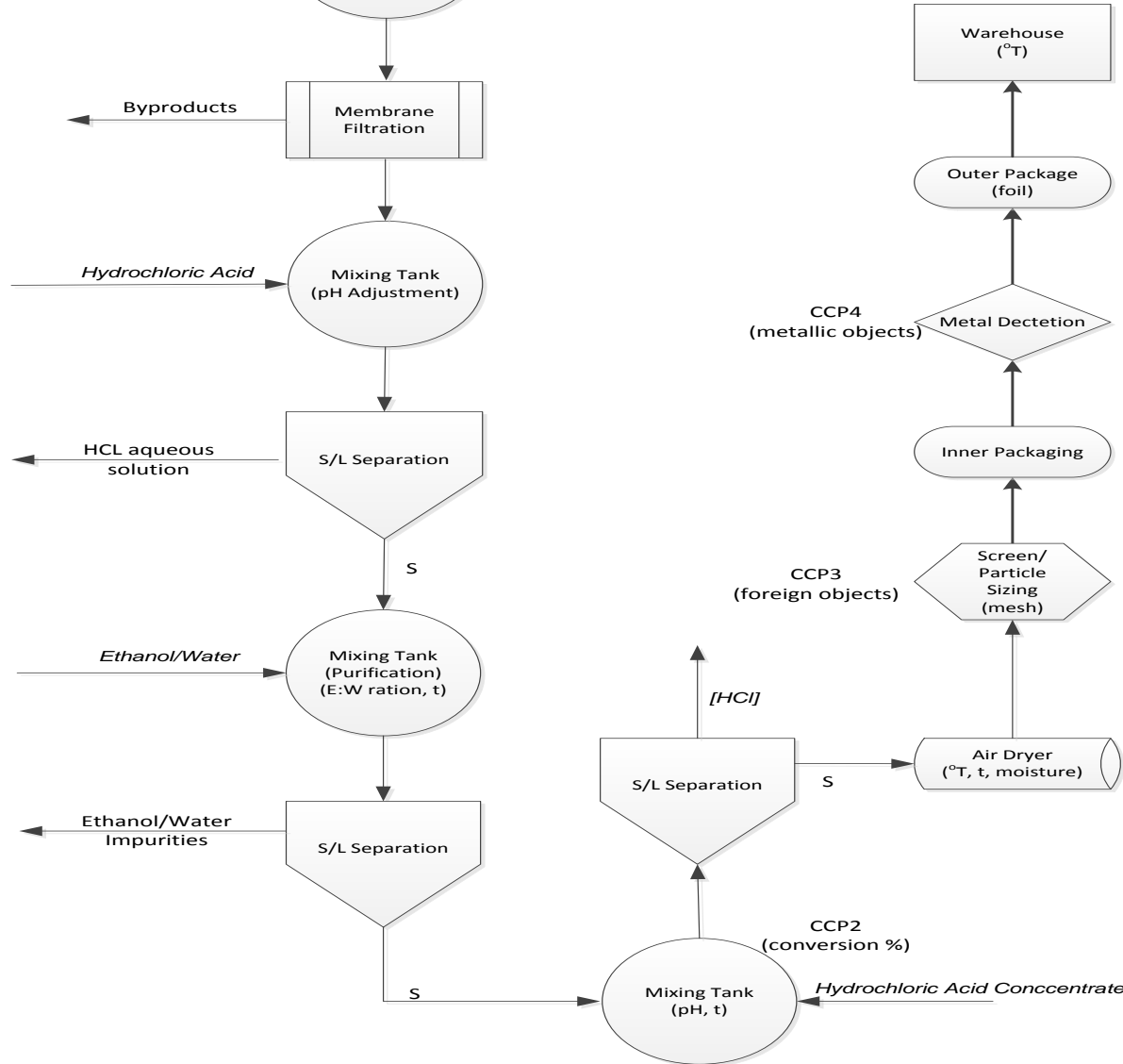


*Shanghai Institute of Materia
Medica (Chinese Academy of
Sciences)*

4,5-Dioxo-4,5-dihydro-1H-pyrrolo[2,3-f]quinolone-2,7,9-tricarboxylic acid 2-ethyl ester 7,9-dimethyl ester

CCP1
(Assay, Heavy Metals)

(A) Purified Water, ...um Hydrochloric
(B) Sodium Chloride, Silicon Dioxide



Chemical structure of PQQ (4,5-dihydro-4,5-dioxo-1H-pyrrolo-[2,3-f]quinoline-2,7,9-tricarboxylic acid) with atom nomenclature.

All carbon and nitrogen atoms of PQQ are derived from conserved tyrosine and glutamate residues of the PqqA peptide.

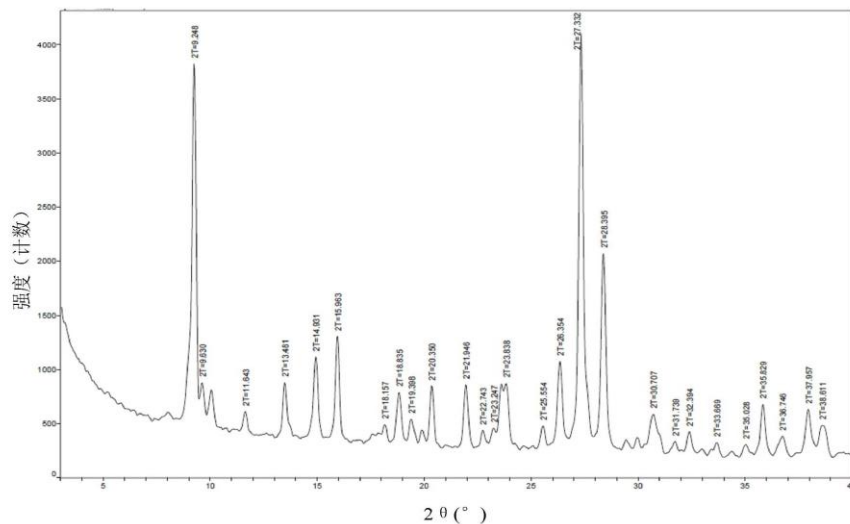
R₁ and R₃ represent the N- and C-terminal portions of PqqA, respectively.

R₂ represent a three-amino-acid linker between Glu and Tyr

- Chemical Synthesis
- Improved Crystal Form

Crystallization remodeling of PQQ

X-ray diffraction



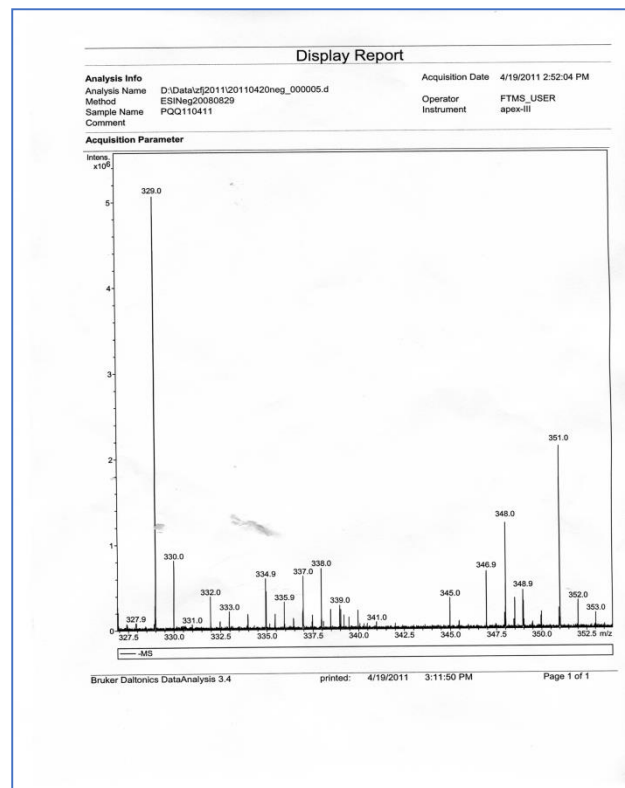
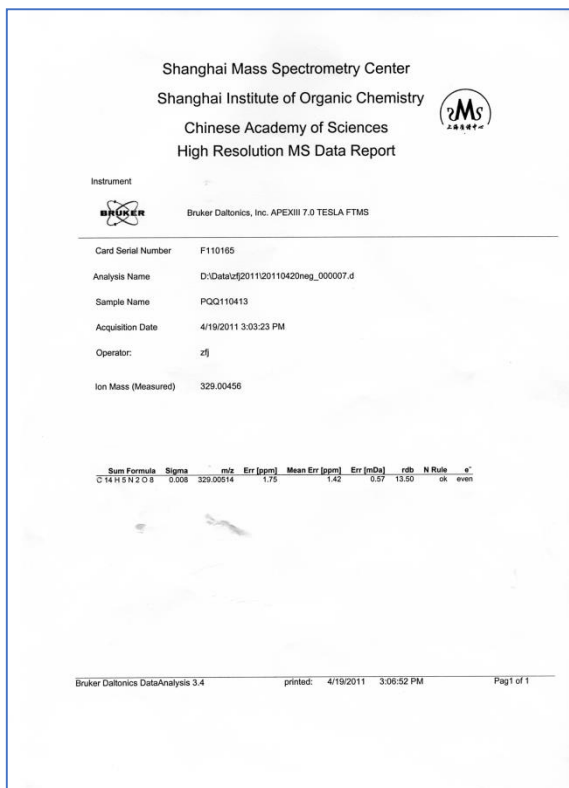
Low hygroscopicity
High stability
Excellent storage
performance
Wide application
prospect

Patent pending (PCT)

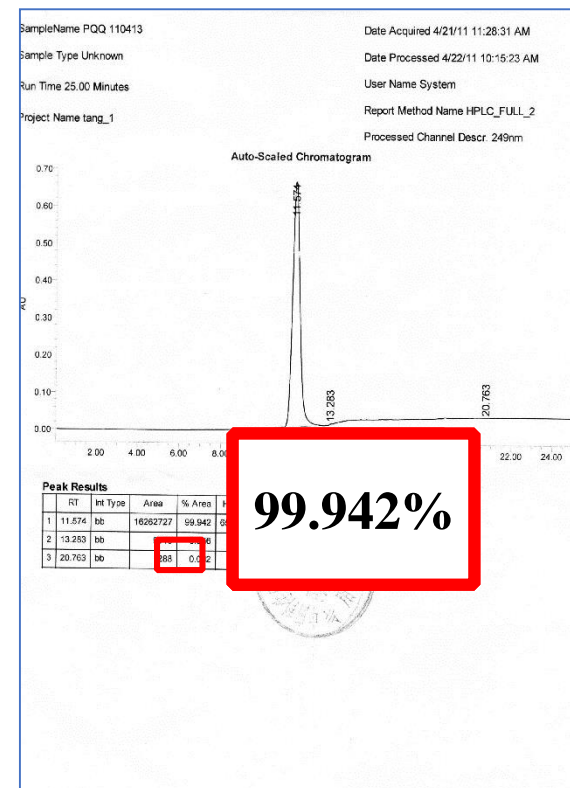
Supported by Shanghai Institute of Materia Medica (Chinese Academy of Sciences)

Excellent Quality and Traceable System

- High Resolution MS Data



- HPLC



PQQ Safety

F2011000002

中国疾病预防控制中心营养与食品安全所

检 验 报 告

样品受理编号: 201105012

样 品 名 称: 吡咯并喹啉醌二钠盐



Third party certification by
authoritative testing organizations in
China

- ☐ Acute toxicity test
- ☐ Ames test
- ☐ Micronucleus test
- ☐ Chromosomal aberration test

[Food Chem Toxicol.](#) 2015 Jan;75:146-50.

A subchronic oral toxicity study on pyrroloquinoline quinone (PQQ) disodium salt in rats.

- A subchronic oral toxicity study on pyrroloquinoline quinone (PQQ) disodium salt was performed in rats.
- Sprague-Dawley rats were randomly divided into four groups (10 rats/sex/group) and administered with PQQ disodium salt at doses of 0 (control), 100, 200 and 400 mg/kg bw/day by gavage for 13 weeks.
- Daily clinical observations and weekly measurement of body weights and food consumption were conducted. Blood samples were obtained on day 46 and day 91 for measurement of hematology and serum biochemical parameters. Animals were euthanized for necropsy, selected organs were weighted and recorded. Histological examination was performed on all tissues from animals in the control and PQQ disodium salt treatment groups.
- No mortality or toxicologically significant changes in clinical signs, body weight, food consumption, necropsy findings or organ weights was observed. Differences between treated and control groups in some hematological and serum biochemical examinations and histopathological examination were not considered treatment-related.
- The no-observed-adverse-effect-level (**NOAEL**) of PQQ disodium salt in rats was considered to be **400 mg/kg** bw/day for both sexes, the highest dose tested.

Self-Affirmation GRAS verified by expert panel.

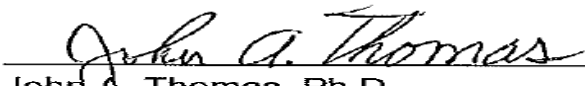
We, the Expert Panel, have independently and collectively critically evaluated the information summarized above and unanimously conclude that there is reasonable certainty that no harm will result from the use and intended use levels of Nascent Health Sciences' PQQ disodium salt. Pyrroloquinoline quinone (PQQ) disodium salt is proposed for use due to its nutritive value in the United States (U.S.) in foods, such as energy, sport, and isotonic drinks; non-milk based meal replacement beverages; water (bottled, enhanced, fortified); milk-based meal replacement beverages; cereal and granola bars; and energy, meal replacement, and fortified bars. PQQ is also intended for use in dietary supplements. PQQ disodium salt is intended to be used in these foods at a maximum level of 20 mg PQQ disodium salt/serving. These proposed uses would result in mean and 90th percentile all-user intakes of 61 and 145 mg/person/day, or 0.9 and 2.1 mg/kg bw/day, respectively. These exposures are greater than 100-fold lower than the NOAEL reported in the 90-day rat study.

Therefore, we conclude that the intended use and use levels of PQQ disodium salt, manufactured according to current Good Manufacturing Practices (cGMP) and meeting the food-grade specifications presented in the dossier, is Generally Recognized as Safe (GRAS) based on scientific procedures.



Robert J. Nicolosi, Ph.D.
Professor Emeritus, Department of Clinical Laboratory
& Nutritional Sciences
University of Massachusetts Lowell, MA

04/13/2015
Date



John A. Thomas, Ph.D.
Adjunct Professor, Department of Pharmacology & Toxicology
Indiana University School of Medicine Indianapolis, IN

4/14/15
Date

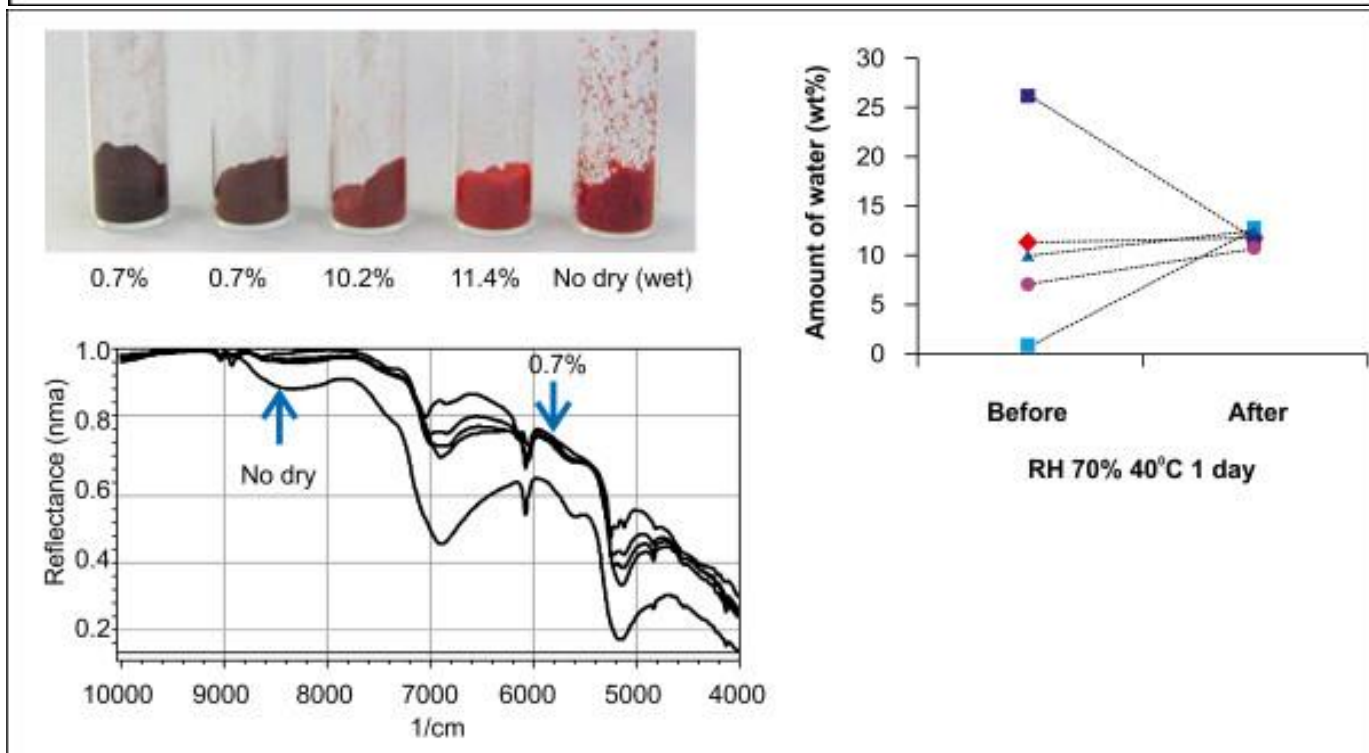
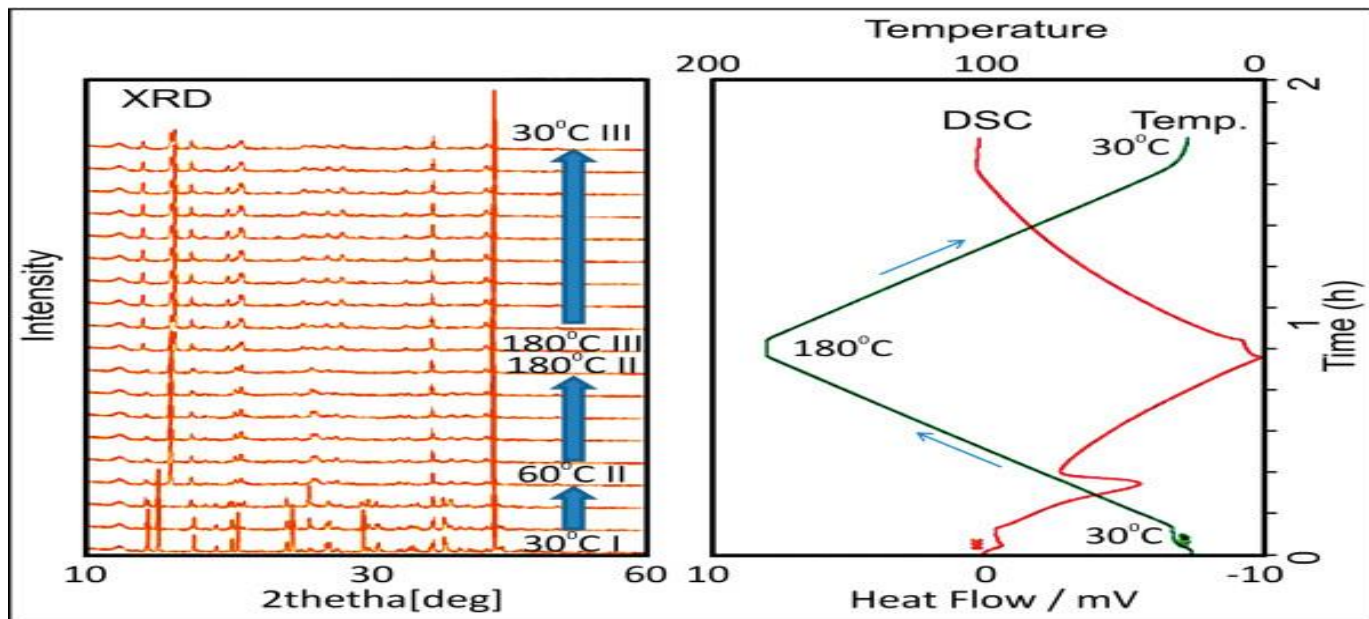
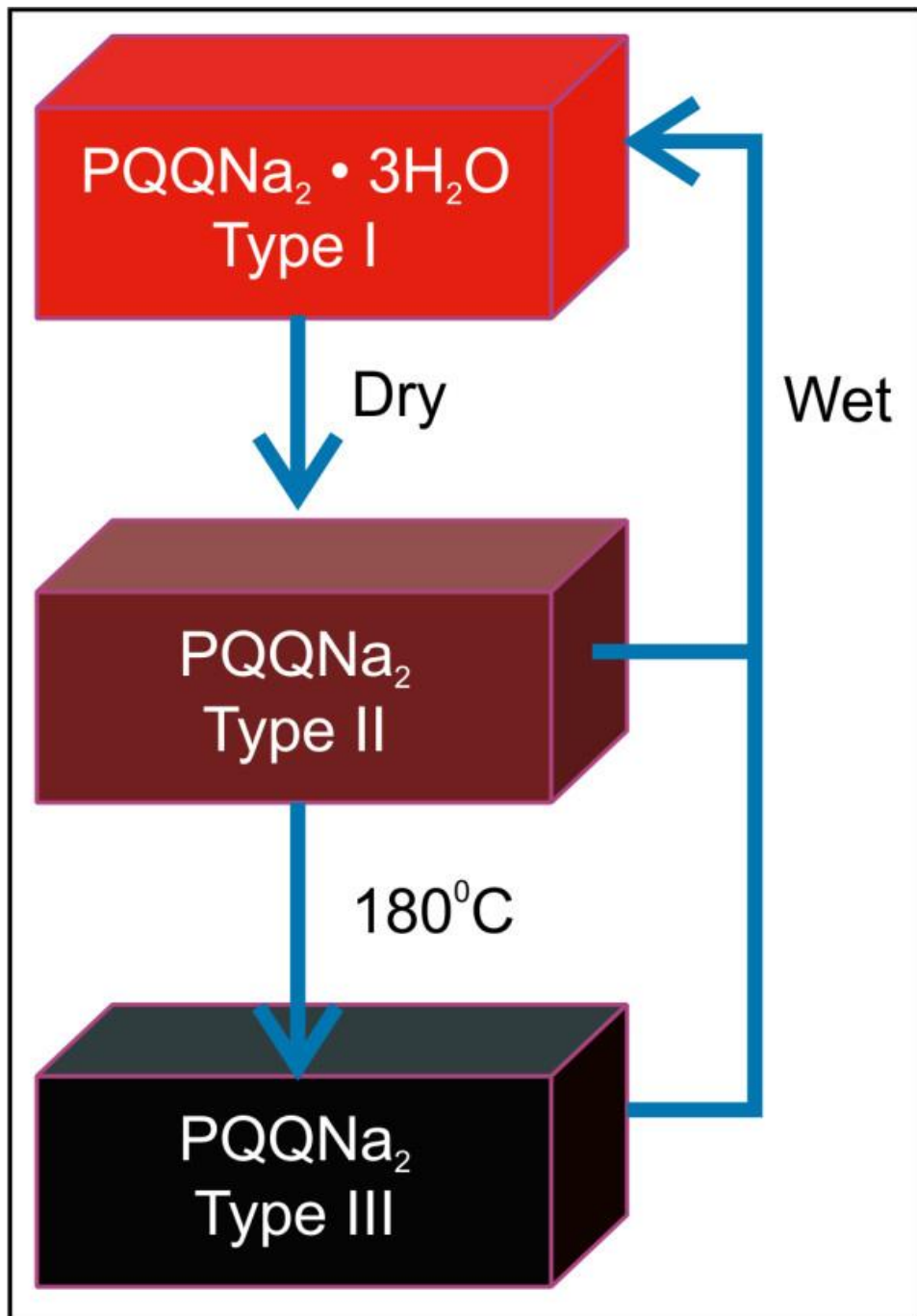


David H. Bechtel, Ph.D., D.A.B.T.
Vice President
Intertek Scientific & Regulatory Consultancy, Bridgewater, NJ

4/15/15
Date

FDA GRAS Approval (GRAS Notice No. GRN 000625) – August 2016

- The subject of the notice is pyrroloquinoline quinone (PQQ) disodium salt. The notice informs FDA of the view of Nascent that PQQ disodium salt is GRAS, through scientific procedures, for use as an ingredient in energy, sport, and isotonic drinks, non-milk based meal replacement beverages, and water (bottled, enhanced, fortified) at a maximum level of 8 milligrams (mg) per serving.
- Based on the information provided by Nascent, as well as other information available to FDA, the agency has no questions at this time regarding Nascent's conclusion that PQQ disodium salt is GRAS under the intended conditions of use.





<i>No.</i>	<i>Patent</i>	<i>Patent Number</i>	<i>Application</i>
<i>1</i>	<i>一种含有吡咯并喹啉醌的强化食品</i>	<i>200810033088.1</i>	<i>Food Additive</i>
<i>2</i>	<i>Purified pyrroloquinoline quinone fortified food</i>	<i>US8,088,422 B2</i>	<i>Food Additive</i>
<i>3</i>	<i>含吡咯并喹啉醌的治疗和预防脂肪肝的药物组合物</i>	<i>2111549.40</i>	<i>Liver Protective</i>

Published Human Studies of PQQ

- Adv Exp Med Biol. 2016;876:319-25. Effect of the Antioxidant Supplement Pyrroloquinoline Quinone Disodium Salt (BioPQQ™) on **Cognitive Functions**.
- Ad Exp Med Biol. 2016;923:215-22. Effects of Antioxidant Supplements (BioPQQ™) on **Cerebral Blood Flow** and Oxygen Metabolism in the Prefrontal Cortex.
- J Nutr Sci Vitaminol (Tokyo). 2015;61(3):241-6. Effects of Orally Administered Pyrroloquinoline Quinone Disodium Salt on **Dry Skin Conditions** in Mice and Healthy Female Subjects.
- J Nutr Sci Vitaminol (Tokyo). 2015;61(3):233-40. Effects of Pyrroloquinoline Quinone Disodium Salt Intake on the **Serum Cholesterol** Levels of Healthy Japanese Adults.
- J Nutr Biochem. 2013 Dec;24(12):2076-84. Dietary pyrroloquinoline quinone (PQQ) alters indicators of **inflammation and mitochondrial-related metabolism** in human subjects.
- Functional Foods in Health and Disease 2012, 2(8):307-324, Effects of Oral Supplementation with Pyrroloquinoline Quinone on **Stress, Fatigue, and Sleep**.
- Medical Consultation and New Remedies, 2011. 48(5):1. Koikeda T et al. Pyrroloquinoline quinone disodium salt **improves higher brain function**.
- Food Style, 2009;13(7):50-3. Nakano M, et al. Effect of pyrroloquinoline quinone (PQQ) on **mental status** of middle-aged and elderly persons.
- Anal Biochem. 1999 May 1;269(2):317-25. Characterization of pyrroloquinoline quinone amino acid derivatives by electrospray ionization mass spectrometry and **detection** in human milk.
- Annu Rev Nutr. 1998;18:145-77. Newly discovered redox cofactors: possible nutritional, medical, and pharmacological relevance to higher animals.
- Biochim Biophys Acta. 1992 Dec 8;1156(1):62-6. **Trace levels of pyrroloquinoline quinone in human** and rat samples detected by gas chromatography/mass spectrometry.
- Life Sci. 1990;47(23):2135-41. Extractions of pyrroloquinoline quinone from crude **biological samples**.
- Biochem J. 1986 Nov 1; 239(3): 789–791. Covalently bound pyrroloquinoline quinone is the organic prosthetic group in **human placental lysyl oxidase**.

Effect of the Antioxidant Supplement Pyrroloquinoline Quinone Disodium Salt (BioPQQ™) on Cognitive Functions.

- A randomized, placebo-controlled, double-blinded study to examine the effect of PQQ disodium salt (BioPQQ™) on cognitive functions was conducted with 41 elderly healthy subjects.
- Subjects were orally given 20 mg of BioPQQ™ per day or placebo, for 12 weeks.
- For cognitive functions, selective attention by the Stroop and reverse Stroop test, and visual-spatial cognitive function by the laptop tablet Touch M, were evaluated.
 - In the Stroop test, the change of Stroop interference ratios (SIs) for the PQQ group was significantly smaller than for the placebo group. In the Touch M test, the stratification analyses dividing each group into two groups showed that only in the lower group of the PQQ group (initial score < 70), did the score significantly increase.
 - Measurements of physiological parameters indicated no abnormal blood or urinary adverse events, nor adverse internal or physical examination findings at any point in the study.
 - The preliminary experiment using near-infrared spectrometry (NIRS) suggests that cerebral blood flow in the prefrontal cortex was increased by the administration of PQQ.
- The results suggest that PQQ can prevent reduction of brain function in aged persons, especially in attention and working memory.

[Ad Exp Med Biol.](#) 2016;923:215-22.

Effects of Antioxidant Supplements (BioPQQ™) on Cerebral Blood Flow and Oxygen Metabolism in the Prefrontal Cortex.

- In the present study, we measured regional cerebral blood flow (rCBF) and oxygen metabolism in prefrontal cortex (PFC), before and after administration of PQQ, using time-resolved near-infrared spectroscopy (tNIRS).
- A total of 20 healthy subjects between 50 and 70 years of age were administered BioPQQ™ (20 mg) or placebo orally once daily for 12 weeks.
- Hemoglobin (Hb) concentration and absolute tissue oxygen saturation (SO₂) in the bilateral PFC were evaluated under resting conditions using tNIRS.
- We found that baseline concentrations of hemoglobin and total hemoglobin in the right PFC significantly increased after administration of PQQ ($p < 0.05$). In addition, decreases in SO₂ level in the PFC were more pronounced in the PQQ group than in the placebo group ($p < 0.05$).
- These results suggest that PQQ causes increased activity in the right PFC associated with increases in rCBF and oxygen metabolism, resulting in enhanced cognitive function.

Dietary pyrroloquinoline quinone (PQQ) alters indicators of inflammation and mitochondrial-related metabolism in human subjects.

- Pyrroloquinoline quinone (PQQ) influences energy-related metabolism and neurologic functions in animals. The mechanism of action involves interactions with cell signaling pathways and mitochondrial function.
- Using a crossover study design, 10 subjects (5 females, 5 males) ingested PQQ added to a fruit-flavored drink in two separate studies.
- In study 1, PQQ was given in a single dose (0.2 mg PQQ/kg). Multiple measurements of plasma and urine PQQ levels and changes in antioxidant potential [based on total peroxyl radical-trapping potential and thiobarbituric acid reactive product (TBAR) assays] were made throughout the period of 48 h.
- In study 2, PQQ was administered as a daily dose (0.3 mg PQQ/kg). After 76 h, measurements included indices of inflammation [plasma C-reactive protein, interleukin (IL)-6 levels], standard clinical indices (e.g., cholesterol, glucose, high-density lipoprotein, low-density lipoprotein, triglycerides, etc.) and (1)H-nuclear magnetic resonance estimates of urinary metabolites related in part to oxidative metabolism.
- Dietary PQQ exposure (Study 1) resulted in apparent changes in antioxidant potential based on malonaldehyde-related TBAR assessments.
- In Study 2, PQQ supplementation resulted in significant decreases in the levels of plasma C-reactive protein, IL-6 and urinary methylated amines such as trimethylamine N-oxide, and changes in urinary metabolites consistent with enhanced mitochondria-related functions.

J Nutr Sci Vitaminol (Tokyo). 2015;61(3):241-6.

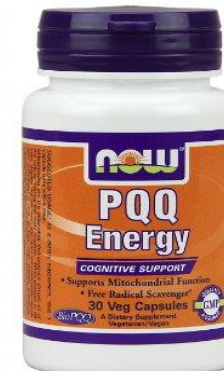
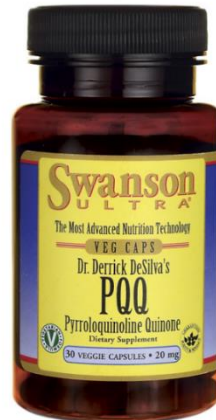
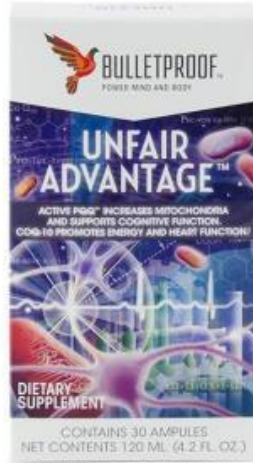
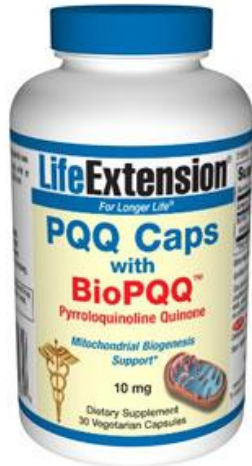
Effects of Orally Administered Pyrroloquinoline Quinone Disodium Salt on **Dry Skin Conditions** in Mice and Healthy Female Subjects.

- The present study aimed to investigate the effects of orally administered PQQ on skin moisture, viscoelasticity, and transepidermal water loss (TEWL) both in dry skin mouse models and in healthy female subjects with a subjective symptom of dry skin.
- In our dry skin mouse model study, oral intake of PQQ (0.0089%, w/w, in the diet for 6 wk) significantly decreased the number of mast cells in the dermis and the number of CD3(+) T-cells in the epidermis.
- In our human study, oral intake of PQQ (**20 mg/d for 8 wk**) significantly inhibited the increase in TEWL on the forearm.
- Subject questionnaires showed positive impressions for the improvement of skin conditions.
- These results suggest that oral intake of **PQQ improves skin conditions** both in female subjects with **dry skin** and in mice with a compromised skin barrier function.

Effects of Pyrroloquinoline Quinone Disodium Salt Intake on the Serum Cholesterol Levels of Healthy Japanese Adults.

- In this study, the effects of PQQ disodium salt on serum TG and cholesterol levels in humans after 6 and 12 wk of treatment at an oral dosage of 20 mg/d were examined.
- This trial was conducted according to a randomized, placebo-controlled, double-blinded protocol.
- A total of 29 healthy Japanese adults, ranging from 40 to 57 y old, with normal to moderately high TG levels (110-300 mg/dL) as measured by a recent blood examination, were included in this study.
- In eleven volunteers out of 29, serum low-density lipoprotein cholesterol (LDL-chol) levels at baseline were high (≥ 140 mg/dL).
- After 12 wk, the mean serum TG levels had not changed; however, a marginally significant decrease in the mean LDL-chol (from 136.1 to 127.0 mg/dL) was observed in the PQQ group.
- In the stratification analysis of the high LDL-chol subgroup (baseline LDL-chol level ≥ 140 mg/dL), the mean LDL-chol levels decreased significantly from the baseline values in the PQQ group compared to the placebo group.
- Our study findings suggest that PQQ suppressed the LDL-chol level, which is an important finding, because a high level of this lipid is a risk factor for various lifestyle-related diseases.

Existing PQQ products on the market



Product display only.
Product may or may not be using PureQQ



60 Capsules of 40mg PQQ (Anti-Aging DNA Therapy)

~~\$19.00~~

\$15.20

120 Capsules of 40mg PQQ (Anti-Aging DNA Therapy)

Best Value Offer

~~\$28.00~~

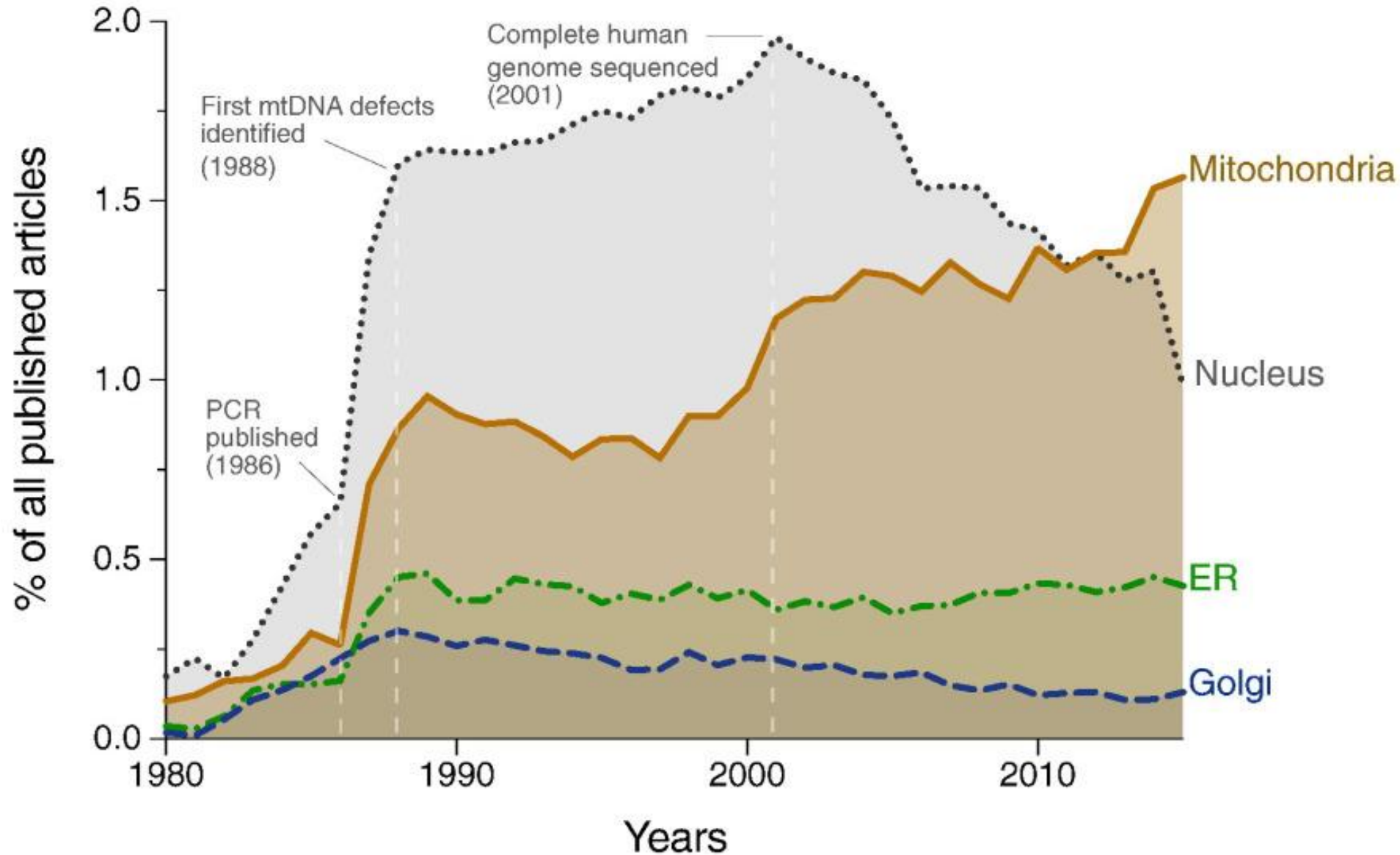
\$22.40 (4.8g of PQQ/bottle - PQQ cost?? \$4200/kg)

**PQQ ANTI-AGING
DNA THERAPY**

Increases MITOCHONDRIAL ENERGY
Combats CELLULAR AGING
Repairs degraded DNA & NERVES

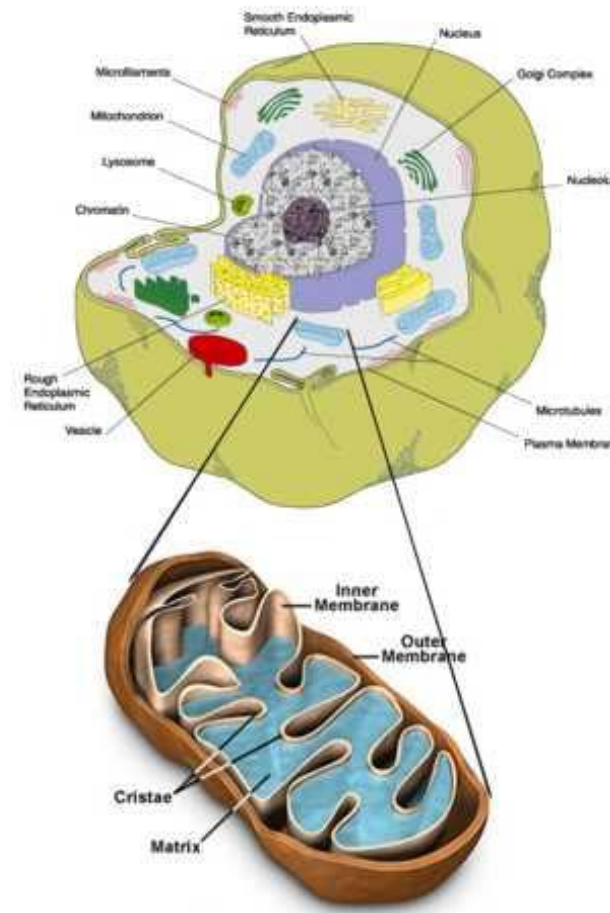
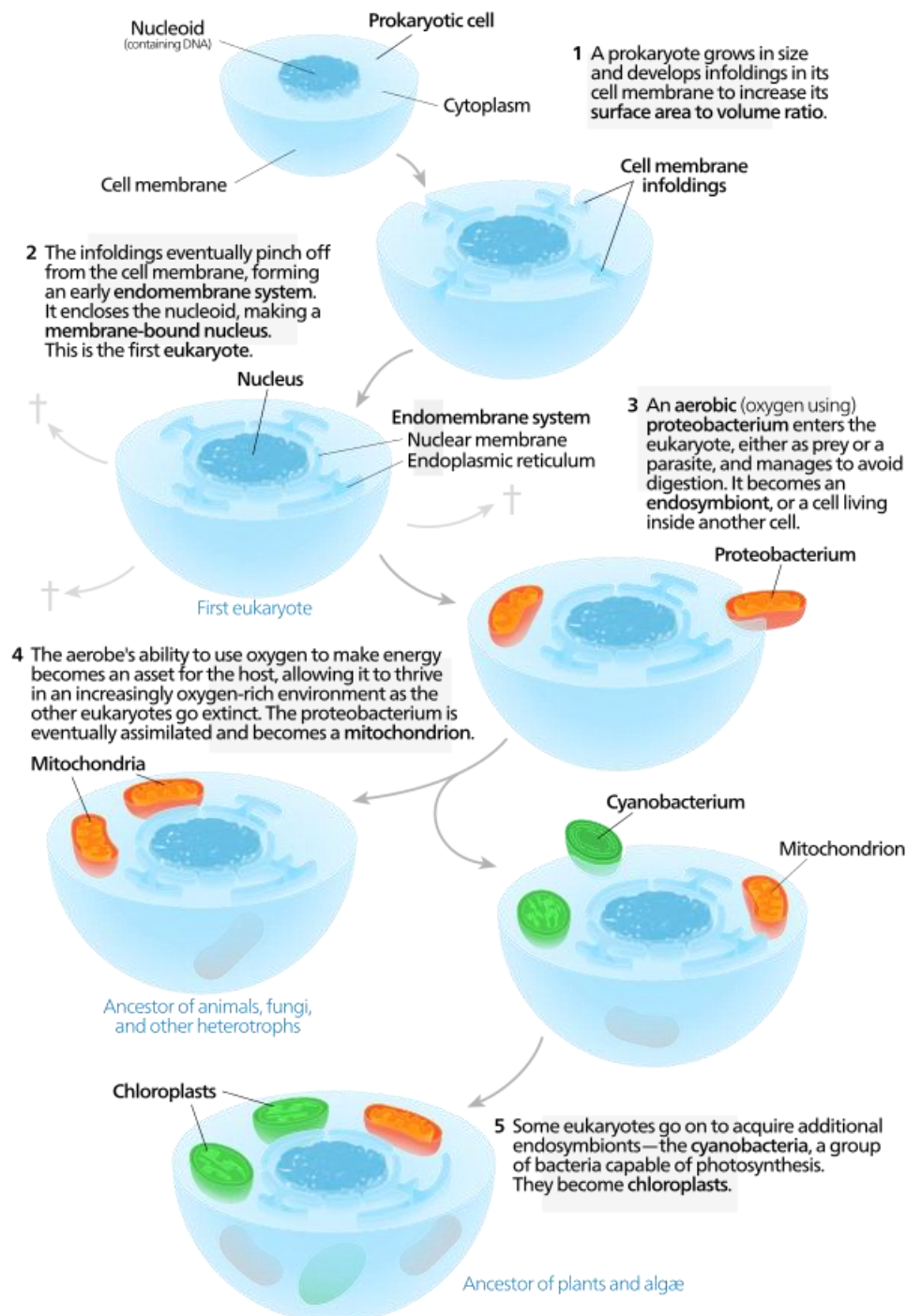
40MG

Growth in Medical Publications



Normalized proportions of published Medline-indexed medical articles from 1980 to January 1, 2016, related to various cellular components: mitochondria, nucleus, endoplasmic reticulum (ER), and Golgi apparatus. Note the increase in mitochondria-related publications following the publication of polymerase chain reaction (PCR) in 1986, the discovery of the first pathogenic mtDNA mutation/deletion in the 1988, and steady rise since the year 2000. In comparison, the number of publications about the cell nucleus has steadily decreased in the 'post-genomic era' following the completion of the human genome project in 2001, which demonstrated that the long searched genetic origin of common chronic diseases is likely not encoded in nuclear genes.

What ARE Mitochondria ?



EndoSymbiotic Bacteria

“The Little Friends Within”

- ☐ Maternally Inherited Co-Incident DNA
- ☐ Aerobic Cellular Respiration (90%)
- ☐ Chloroplast's Gas Exchanger ($O_2 \leftrightarrow CO_2$)
- ☐ Signaling System

The “ PowerHouses of the Cell ”

- ☐ Oxidative - Phosphorylative Coupling
- ☐ Endothermic Heat Source
- ☐ Generation of ATP